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H. C. Olson
Iowa State College

B. W. Hammer
Iowa State College

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Bacteriology of Butter

V. Studies on the Microorganisms in Churns

By H. C. Olson and B. W. Hammer

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE
AND MECHANIC ARTS

R. M. Hughes, Acting Director

DAIRY INDUSTRY SECTION

AMES, IOWA

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Bacteriology of Butter

V. Studies on the Microorganisms in Churns*

By H. C. Olson and B. W. Hammer

The utensils and equipment with which dairy products come in contact on farms and in dairy plants constitute a very important source of the microorganisms in these materials. Because their construction makes them especially difficult to clean, certain pieces of equipment are of much greater significance in this connection than others. In butter plants the churns are a particularly important source of organisms and, moreover, this contamination occurs subsequent to the pasteurization of the cream so that none of the organisms added by it are destroyed.

The growth of the organisms present in butter is delayed or prevented by the salt that is commonly used in the making but, nevertheless, the direct action of organisms is frequently responsible for deterioration in this product. Undoubtedly, bacterial deterioration of butter is becoming of relatively greater significance as the marketing of unsalted and low salted butter increases and as chemical deterioration is more completely controlled. Because of the increasing importance of the contamination of butter, attempts to control it are being given more attention than formerly.

The work herein reported was carried out at the Iowa Agricultural Experiment Station in a study of the microbiological condition of churns. The data are presented in seven parts as follows:

Part 1. Extent of the contamination of churns in commercial use.
Part 2. Regular treatment of churns with hot water.
Part 3. Use of chemicals on churns treated with hot water.
Part 4. Treatment of highly contaminated churns.
Part 5. Contamination of churns from the air.
Part 6. General observations on the contamination from churns.
Part 7. Influence of the contamination from churns on the keeping qualities of butter.

GENERAL CONSIDERATIONS

The proper care of the churn presents one of the most difficult microbiological problems in a butter plant. The fundamental reason for this is that the churn is ordinarily made of

* Project No. 126 of the Iowa Agricultural Experiment Station. The studies on the microorganisms in churns were financed, in large part, by a grant from the Iowa State Brand Creameries, Inc., of Mason City, Iowa.

wood and, accordingly, the surface exposed to the cream and to the butter is much more difficult to keep in a satisfactory condition than if it were metal. The surface of the wood is more or less irregular, the irregularities are increased as the wood takes up water and the wood may sliver or crack so that the mechanical removal of organisms and milk solids is greatly hindered. The pieces of wood cannot be fitted so closely that curd, fat, etc., never get between them. The wood also tends to soak up milk solids that cannot be removed readily and these may supply nutrients for the growth of organisms; certain species, especially some of the molds, can undoubtedly secure the nutrients necessary for their development from the wood itself. Heat penetrates the wood very slowly so that the organisms in joints, cracks and irregularities in the surface are not easily destroyed by heat treatment and the churn does not dry as rapidly after draining as is desirable. The points where drying is especially slow may be points at which milk solids tend to accumulate so that conditions satisfactory for the growth of microorganisms are developed. The protection of organisms in a churn also interferes with their destruction by chemicals.

When the equipment required for the working of the butter was placed in the churn—that is when the combined churn and worker was developed—the problem of properly caring for the churn was further complicated because various parts became very inaccessible. Moreover, this development introduced working parts into the interior of the churn, and these included bearing surfaces where milk solids may accumulate and where drying may be very slow, especially after the inevitable wear has taken place.

The heavy loads that a churn must carry throw a strain on the parts and eventually tend to open the joints in the wood, loosen bolts, etc., and thus provide additional points at which milk solids and moisture may collect and microorganisms develop. The strain on the churn may be especially serious during the working of the butter.

With the churn, as with most other pieces of dairy plant equipment, the object of the treatment given should be to so control the numbers of organisms present that the contamination from this source is comparatively unimportant. Since butter is certain to contain organisms from the cream, the piping, the air, etc., there is little reason to control the contamination from the churn beyond a certain point. If a churn could be sterilized, it would quickly be recontaminated from the cream, the water used to wash the butter, the air, etc. The essential point is to control the organisms in a churn so that they will not be a factor in the deterioration of the butter. In considering the contamination from churns it should be recognized that

many of the organisms in the cream are carried away in the buttermilk and, accordingly, are of no significance from the standpoint of the deterioration of the butter; the organisms in the buttermilk undoubtedly include many of those coming from the churn.

The irregular distribution of organisms in a churn greatly complicates the study of the microbiological condition of the churn. If a rinse procedure is used the organisms cannot all be picked up in the rinse material and, undoubtedly, from some parts of the churn, especially where fat and curd have accumulated, very few of the organisms are secured. It is probable that from certain points the organisms are forced out only when the churn is under a severe strain, for example when butter is being worked, and are well protected at other times. The agar disc method can be used only on certain parts of the churn, and these do not include the points where the growth of organisms is most likely to occur. Moreover, not all of the organisms are picked up from the area on which an agar disc is prepared (7).

REVIEW OF LITERATURE

Lund (12) attributed the presence of large numbers of yeasts and molds in pasteurized cream butter to contamination following pasteurization, especially from the churn, because he had previously found (11) that yeasts and molds are killed by proper pasteurization. He stated (13) that infected churns are an important source of yeasts and molds in butter and that neither treatment with chloride of lime nor with "scalding hot" water is effective in removing the contamination. Treatment with hot alkali solution followed by hot, freshly prepared milk of lime, along with occasional repacking of stuffing boxes, was effective in reducing the yeast count on the butter to less than 10 per ml. In a study of 337 lots of Canadian butter, Lund (13) found that the majority of the mold counts were below 10 per ml., while the majority of the yeast counts were 1000 per ml. or over. He stated, "Where extensive recontamination of pasteurized cream with yeasts occurs it is altogether probable that even heavier recontamination by bacteria takes place at the same time." Later, Lund (14) reported data on 537 lots of "Storch negative" Canadian butter which showed that over 60 percent of them had yeast counts of more than 1000 per ml. His investigations led him to conclude that, where high yeast counts occurred regularly, 9 times out of 10 the trouble was due to an infected churn. Lund also noted a distinct correlation between high yeast counts and high bacterial counts on butter made without starter. The bacteria were always much more numerous than the yeasts and molds.

The work of Gregory (3) shows that churns may be an important source of contamination of butter and that ordinary cleaning methods do not eliminate this contamination. He determined the amount of infection remaining after various cleaning treatments by rinsing the churn with 200 gallons of water for 30 minutes and determining, by plate counts, the number of organisms added by the churn. There were considerable numbers of organisms in the churn after each of six different treatments, but after some of them the numbers were comparatively small. In the trials in which the churn was washed, allowed to stand overnight and then washed again and treated with a commercial germicide, the counts were not only low but were exceedingly uniform. Overnight treatment with a suspension of lime after hot water treatment gave unsatisfactory results; the only advantage of the lime was that it improved the odor of the churn. The data show that the bacteria in the churns were far more numerous than the acid-tolerant organisms which were largely yeasts and molds.

Bouska and Brown (1) reported that the churn may be an important source of yeasts and oidia, unless it is carefully treated, and that more organisms were added to the butter during the working than during the churning because of the organisms being forced out of the working parts of the churn. They noted a rather close correlation between the yeast and mold counts on butter and the keeping quality.

Stiritz (24) suggested that yeast and mold counts should be used as an index to the whole buttermaking process, rather than to the pasteurization only, because of the possibilities of contamination of the cream subsequent to pasteurization, especially from the churn.

The importance of churn sanitation in relation to the keeping quality of butter is emphasized by the work of Shutt (22). From a study of 21 samples of pasteurized cream butter held in cold storage at 10° F. for about 6 months, it appeared that butter made from pasteurized cream churned with the least possible contamination held its grade better than butter made from pasteurized cream contaminated during the churning process. In a later report Shutt (23) presented data showing the extensive contamination from churns and the effectiveness of various cleaning treatments in reducing this contamination. He used several churns of from 1 to 8-gallon capacities, all excessively contaminated by sour cream, and determined the contamination in the churns by rinsing them with peptone water and plating it. A large number of organisms was found to be removed mechanically by successive rinsings with cold water. Considerable contamination remained after treatment with boiling "lime solution" for 5 minutes, diluting this to the

capacity of the churn and holding for 3 days; but by heating the "lime solution" to boiling for 30 minutes before diluting the contamination was greatly reduced. Treatment of the churns with 0.3 percent sulphuric acid reduced the number of organisms but the results were not satisfactory. Shutt found that a solution of 7 grams of "Sterilac" in 1 gallon of cold water reduced the contamination in a 3-gallon churn very siginificantly with either a 5-minute or a 30-minute exposure. When used cold a 2 percent solution of "Montanin" exposed to the churn for 30 minutes was ineffective but when used hot there was a marked decrease in both bacteria and molds, especially in the latter. Due to the unsuitability of the churn used the results with hyperactive iodine, in concentrations ranging from 0.010 to 0.229 percent, were inconsistent. The data secured with a different churn, however, showed that hyperactive iodine had a decided germicidal effect on bacteria and molds. Hyperactive iodine was found to penetrate fat readily, which is an important point in the treatment of "greasy" churns.

Ruehle (20), in a general discussion, emphasized the difficulties involved in churn sanitation and advocated the frequent "sterilization" of churns in constant use. Soaking for 3 days in milk of lime, followed by several rinsings with hot water, was suggested for new churns and old ones which had stood idle.

Haglund, Barthel and Waller (5) studied four churn cleaning methods: these were (1) hot water, (2) hot water and a coating of lime, (3) hot milk of lime, and (4) water heated to boiling with steam. The last method was the most effective in that butter comparatively free from yeasts and molds could be produced with it. The need for regular treatment of churns was emphasized by the fact that the churns became infected again after standing for from 3 to 5 days. In 14 trials butter from "sterilized" churns showed slightly better keeping qualities than butter from infected churns, the average difference being 0.9 points after storage for 10 days and 1.6 points after storage for 20 days. In a series of tests at 14 well-managed dairies, no definite improvement in the butter or in its keeping qualities resulted from the introduction of modifications of the churn cleaning methods then used in these dairies.

According to Hood and White (8) yeasts and molds in cream are all destroyed by pasteurizing at a temperature of 185° F. for 10 minutes. These investigators stated that the churn is the most troublesome source of contamination of pasteurized cream, but that the contamination from the churn is not serious if a comparatively new churn is used and liming practiced regularly.

Quam (19) compared the efficiencies of chemical sterilizers

and of ordinary methods, involving the use of hot water and hot milk of lime, for the treatment of churns. The extent of contamination of the churns was estimated by adding 10 liters of sterile water to the churn, agitating for 5 minutes and determining, by plate counts, the number of organisms added to the water. The data show that after the hot water and hot milk of lime treatment considerable numbers of organisms remained in the churns; a significant reduction in organisms was effected when this method was followed by a 5-minute exposure to 10-gallons of water, containing one-half ounce of "Diversol" per gallon, at ordinary temperature. Still better results were secured when "Diversol" was used as a sole means of treating the churns; it was employed at the rate of 1 ounce per gallon in 50 gallons of water at temperatures from 120° to 125° F., the exposure being for 5 minutes. The best results were obtained by using a chlorinated lime solution after treatment with hot water and hot milk of lime; when a concentration of 6 ounces of chlorinated lime in 10 gallons of water at ordinary temperatures was used the contamination was negligible. In all the trials the numbers of bacteria were much greater than the numbers of yeasts and molds, both before and after treatment.

Groth (4) stated that churns are the greatest source of recontamination of pasteurized cream but that, if proper washing procedures are used, it is possible to keep them practically free from yeasts and molds.

The results obtained by Macy and Combs (15) indicate that raw cream, pipes, pumps and churns are the most serious potential sources of molds in butter, the churn being particularly important because it is difficult to sterilize. The necessity for the regular treatment of churns was emphasized by the fact that butter made in a churn which had stood idle for a few weeks molded badly in storage. Infected churns, if in good condition, usually responded readily to careful treatment.

Schmidt (21) gives an account of Swedish and Danish experiments on churn treatment. In the Swedish experiments the churns were cleaned with hot water in the customary manner and counts made on the hot rinse water at the end of the exposures. The counts ranged from 5,300 to 876,000 bacteria per ml., showing that considerable numbers of bacteria survived the treatment. In the Danish experiments five methods of cleaning were used and counts were made on the butter and buttermilk from the churns. The data show that only treatment with water heated to boiling with steam was effective in destroying harmful bacteria. After storage for 10 days the butter made in a carefully treated churn scored from 0.0 to 3.5 points higher than the butter made in a churn cleaned by ordinary methods and after 20 days storage the differences in scores ranged from 1.0 to 6.5 points.

From his work on churn sanitation James (9) concluded that the churn may be a source of contamination of butter and that it is difficult to entirely eliminate this contamination. He estimated the extent of the contamination of a churn by rinsing with 12 gallons of sterile water and plating this. Churns which had been treated with hot water and washing powder were found to contain many yeasts and molds; no bacterial counts were run in this series. In a second series, bacterial counts, as well as yeast and mold counts, were run in 12 of the 16 trials. In these 12 trials, in which heat or chemical treatment was used, the churn was rendered yeast and mold-free seven times but it always contained some bacteria. Increase in temperature and in period of exposure greatly favored the chemical treatment and, as a whole, this type of treatment was rather successful. Temperature of medium, fullness of churn and length of exposure were apparently the cardinal factors in churn cleaning. There was evidence of organisms being lodged in inaccessible places, and James suggested that churns are not rendered sterile because the sterilizing medium does not come in contact with the organisms. That the organisms are killed when in direct contact with chemical sterilizers was shown by a third series of experiments in which suspensions of organisms derived from churns were largely destroyed by exposure to ordinary dilutions of commercial germicides.

The possibility of the growth of yeasts and molds on the wood of churns was shown by Libbert (10). He isolated 57 yeast and mold cultures from various churns and all except 3 of the yeast cultures grew on a medium containing water, agar and ground fir wood; the organisms also grew on this medium minus the fir wood, but the cultures died within 10 days whereas those on the fir wood medium remained viable for at least 6 weeks. Libbert found evidence of the accumulation of debris and of the growth of organisms in the joints between the staves of a churn.

The difficulty of rendering churns sterile is emphasized by the work of Morrison, Macy and Combs (18) on the efficiencies of various churn cleaning methods. They used a churn having a high natural contamination which, in addition, was contaminated excessively with broth cultures of species of molds commonly found in churns. The extent of contamination was determined by rinsing the churn before treatment with 3 gallons of tap water, rinsing after treatment with 1 gallon of sterile skimmilk and plating these materials on whey agar; the plates for mold counts were acidulated with tartaric acid. "The results were not recorded quantitatively, but qualitatively, to give a relative idea of the amount of infection." In five trials with hot water treatment, the churn being one-sixth to one-third full, tem-

peratures of 85° to 88° C. (185.0° to 190.4° F.) and exposures of from 5 to 60 minutes were ineffective in reducing either the mold or bacterial infection. In one trial in which the churn was filled one-third full of water at 96° C. (204.8° F.) and exposed for a period of 30 minutes, the mold infection was entirely eliminated but there was no apparent reduction in bacteria. In three trials in which the churn was filled full of water at temperatures over 97° C. (206.6° F.) and exposed for 3 hours, the molds were entirely eliminated and the bacteria appreciably decreased. It was apparent that regular treatment is essential since the churn regained its mold infection after standing idle for several days. In four steam treatment trials, in which the steam was allowed to flow slowly into the churn for a period of 3 hours, the molds were entirely eliminated and, on the third consecutive day of treatment, the bacteria were markedly decreased. Normal fructification of the molds began again after a few days. Morrison, Macy and Combs reported that, in four trials, sodium hypochlorite solutions used in chlorine concentrations from 35 to 265 ppm., for periods from ½ to 18 hours and at temperatures from 10° to 15.6° C. (50° to 60.1° F.) were ineffective in reducing either the mold or bacterial infection. In four trials with solutions of an "alkaline crystalline hypochlorite," using nearly the same concentrations and exposures as with the sodium hypochlorite, the molds were effectively reduced in only one trial and in none of the trials was there a significant reduction in bacteria. In four trials with chloramine-T solutions the chlorine concentrations ranged from 46 to 350 ppm., the temperatures from 48° to 51.6° C. (118.4° to 124.9° F.) and the exposures were 2 hours in two trials and 18 hours in the other two; in only one trial was there a significant reduction in molds and the bacteria were not significantly reduced in any of the trials. The general results indicated that the bacteria are much more difficult to eliminate than the molds. According to the results secured by Morrison, Macy and Combs, sufficient exposures to hot water and sufficient exposures to flowing steam were the most satisfactory methods for the treatment of churns. These investigators state, "Solutions of sodium hypochlorite, alkaline crystalline hypochlorite and chloramine-T were ineffective." In studies on the rate of heat penetration of wood Morrison, Macy and Combs found that when a churn was exposed to boiling water it required a period of 1½ hours for heat to penetrate the wood to a depth of 1⅜ inches and raise the temperature to 62.3° C. (144.1° F.).

By dismantling two churns, which had been in service for a number of years, and culturing samples of scrapings, wood, etc., taken from various points, Macy, Combs and Morrison (16) demonstrated that numerous infection foci exist in a churn. Of

the 230 samples from one of the churns, 192 carried molds while of the 73 samples from the other churn, only 22 showed mold infection and, in general, the infection was not heavy. The first churn had been receiving ordinary treatment while the second had been receiving careful treatment. The investigators state, "While this report does not concern itself with yeasts or bacteria in the churn, it might be mentioned that the former were consistently present in the great majority of cases and the latter always, sometimes in large numbers."

Coulter (2) reported that the churn was the most important source of contamination of pasteurized cream butter, particularly with yeasts or molds. "Salting the churn" proved very effective in reducing the yeast and mold counts of butter. The procedure used was as follows: Water was added to 20 or 30 pounds of salt until a "thin paste" was secured, the mixture heated to boiling and added to the churn; the churn was revolved 10 or 15 times and then drained, leaving a thin coat of salt crystals on the surface. During a period of 6 months previous to the introduction of this treatment, only 29 percent of the yeast counts on the butter were 0 per ml. while during a year when the treatment was used 86 percent of the yeast counts were 0 per ml. An increase in the percentage of mold counts that were 0 per ml. from 76 to 89 was secured when the salt treatment was used.

The importance of the churn in the contamination of pasteurized cream butter is emphasized by the work of Macy, Coulter and Combs (17). By plating water used to rinse the pipes and pump and also water used to rinse the churn just before use, they showed that the churn contributed much larger numbers of yeasts and molds than did the pipes and pump; no bacterial counts were made. They found a rather close correlation between high mold counts on the churn rinse and high mold counts on the cream after it had been revolved in the churn for 3 minutes. A comparable relationship was noted with the yeasts but was not so definite as with the molds. The churn evidently contributed considerable numbers of bacteria in many of the trials but, since there were always rather large numbers of bacteria and very few yeasts and molds in the pasteurized cream, contamination with bacteria could not be detected as easily as contamination with yeasts and molds. There was evidence that yeasts and molds were being dislodged from the churn during the working process. Macy, Coulter and Combs exposed acidulated whey agar plates to the creamery air for 10 minutes and found that considerable numbers of yeasts and molds fell on the plates during this period. The counts were higher during the summer months than during the remainder of the year.

METHODS

(a) METHODS FOR DETERMINING THE MICROBIOLOGICAL CONDITION OF CHURNS

1. AGAR DISC METHOD

The agar disc method has been described in detail by Hammer and Olson (7). It consists of allowing about 10 ml. of beef infusion agar, containing 2.5 percent air dried agar, to solidify in contact with the surface to be studied, the transferring of the disc thus formed to a sterile petri dish and, finally, the counting of the colonies that develop on incubation, the results being expressed as the number of colonies per square centimeter; for yeast and mold counts, an acidulated agar is employed. Just before pouring the agar, the surface to be covered is moistened with sterile water in order to control the size of the disc and to facilitate its removal.

From two to seven (usually four or five) beef infusion agar discs were prepared for the bacterial counts and, commonly, two whey agar discs (the medium containing 2.5 percent air dried agar and being acidulated to pH 3.5 with lactic acid) for the yeast and mold counts. With chlorine treated churns sterile litmus milk was used to moisten the surfaces to be examined in an attempt to prevent any action of the chlorine on the organisms. The plates were incubated at room temperature, which was commonly about 70° F.; when the counts were low the plates were counted after 4 days, but when the counts were high the plates were counted earlier. The counting was done with the aid of a hand lens. With comparatively few colonies on the plates at least 20 square centimeters were counted, with a great many colonies only a fraction of a square centimeter was counted and with intermediate numbers the area counted varied. Counts of over 400 per sq. cm. were estimated, and counts of 1,000 or more per sq. cm. were estimated as minimum values and recorded as more than 1,000, more than 2,000, etc. The estimates were used as actual counts in calculating averages. The bacterial counts undoubtedly included an occasional yeast since the colonies were not examined in detail but the numbers of yeasts must have been very small.

2. RINSE METHOD

The procedure used in examining churns by the rinse method was as follows: Ten gallons of water were added to the churn and the churn revolved in high gear for 10 minutes. Originally the water was heated nearly to boiling with steam and allowed to cool overnight before use, but later ordinary tap water, on which the counts were consistently low, was employed. The rinse water was plated, before and after exposure, on beef infusion agar for the bacterial counts and on malt agar acidulated

to pH 3.5 with lactic acid for the yeast and mold counts. The water was plated immediately, except in some of the examinations of churns before treatment when it was iced and held for not more than 4 hours. The plates were incubated at room temperature, which was commonly about 70° F., for 4 days unless the molds were very numerous when it was necessary to count the yeast and mold plates after 2 days; the counting was done with a hand lens. The results were expressed as the number of organisms per ml., using 10 gallons of water per churn. In plating the water used to rinse a churn following chlorine treatment and also in plating the water to which chlorine had been added, sterile litmus milk was used for diluting the samples in order to dissipate any residual chlorine. Since detailed examinations of the individual colonies on the beef infusion agar plates were not made the bacterial counts undoubtedly included some yeasts, but the numbers of these were generally very small as compared with the numbers of bacteria.

(b) METHOD FOR DETERMINING THE AVAILABLE CHLORINE CONCENTRATIONS IN CHLORINE SOLUTIONS

The method used for the available chlorine determinations was as follows: A 50 ml. sample of the chlorine solution was mixed with 200 ml. distilled water and 10 ml. of a 15 percent potassium iodide solution, 5 ml. of concentrated HCl (sp. gr. 1.18) were added and mixed with the solution and, after 2 minutes, the iodine liberated was titrated against hundredth normal sodium thiosulfate, using 1 ml. of a 1 percent starch paste as an indicator, near the end of the titration.

EXPERIMENTAL

PART 1. EXTENT OF THE CONTAMINATION OF CHURNS IN COMMERCIAL USE

The extent of the contamination of 27 churns in commercial use in 24 Iowa butter plants was studied by means of agar disc counts. Some of the churns were examined twice, the total number of examinations being 32. The results obtained are presented in table I; a statement of the general types of bacteria present on the discs from each churn is also included.

The bacterial counts on the churns varied from 1.3 to more than 2,000 per sq. cm. Ten of the counts, representing nine churns, were less than 10 per sq. cm.; commonly the bacteria belonged chiefly to the genus Bacillus, but in some instances micrococci were also present. The general condition of the churns at the time the discs were prepared indicated that all of them were being given careful treatment. Seven of the counts were between 10 to 49 per sq. cm. and the bacteria usually included several types, although organisms belonging to the genus Bacillus and micrococci predominated. The churns on

TABLE I. AGAR DISC COUNTS AND BACTERIAL TYPES OF CHURNS IN COMMERCIAL USE

Churn designation*	Date of exam.	Organisms per sq. cm.			Bacterial types present
		Bacteria	Yeasts	Molds	
Aa	11/ 7/30	1.7			Several types but Bacillus types predominant
Aa	4/13/31	6.5	.03	.30	Almost exclusively Bacillus types
Ab	4/13/31	8.3	.13	1.70	Several types but Bacillus types predominant
B	11/ 7/30	63			Many types with yellow micrococci predominant
B	4/13/31	307	1.20	2.20	Many types with yellow and white micrococci predominant; Bacillus types numerous
C	11/ 7/30	228			Many types with micrococci predominant
C	4/13/31	36	.13	.66	Several types but Bacillus types predominant; some micrococci
D	11/ 7/30	7.8			Many types but micrococci predomidant
D	4/13/31	20	.30	3.73	Yellow micrococci predominant; some Bacillus types
E	11/ 7/30	9.2			Mainly Bacillus types
F	11/28/30	>2000			Yellow micrococci predominant; some Bacillus types
G	11/28/30	>2000			Mainly yellow micrococci
H	11/28/30	>1000			Mainly yellow micrococci
I	11/28/30	64	.06	.36	Mainly white micrococci
J	11/28/30	3.3	.07	.28	Very few types with yellow micrococci predominant
Ka	11/28/30	21	.35	.44	Several types with yellow micrococci predominant
Kb	11/28/30	8.9	.45	1.23	Few types with micrococci predominant
L	3/ 2/31	800			Several types with yellow micrococci predominant; some Bacillus types
L	4/13/31	850			Several types with yellow micrococci numerous; some Bacillus types
M	3/ 2/31	>2000			Several types with yellow micrococci predominant
N	3/ 2/31	490			Many types
O	3/ 2/31	37	.15	1.28	Several types with Bacillus types predominant
P	3/23/31	730			Several types with Bacillus types predominant
Q	3/23/31	68	.12	.06	Several types with yellow micrococci predominant; some Bacillus types
R	3/23/31	1.3	.02	.09	Mainly Bacillus types and yellow micrococci
S	3/23/31	32	.04	.36	Several types with yellow micrococci predominant
T	3/23/31	297	.03	.10	Several types with micrococci predominant
U	5/18/31	>1200	.10	.20	Several types with yellow micrococci predominant
V	5/18/31	9.6	0	5.46	Few types with Bacillus types predominant
W	5/18/31	3.5	0	.25	Few types with Bacillus types predominant
Xa	5/18/31	47	.30	5.40	Mainly Bacillus types
Xb	5/18/31	33	.31	3.33	Few types with Bacillus types predominant

* The capital letters designate the creameries and the small letters designate the churns in creameries with more than one churn.

which these counts were secured appeared to be receiving rather careful treatment. Four of the counts were between 50

and 249 per sq. cm., with the bacteria usually including a number of types, although yellow micrococci predominated. Three of the churns yielding these counts were apparently being cleaned rather thoroughly while the other one was not. Six of the bacterial counts were between 250 and 999 per sq. cm.; the bacteria usually included many types with yellow micrococci predominating, but in one churn Bacillus types predominated. The appearance of all of the churns on which these high counts were secured suggested careless treatment and in three of them a slight objectionable odor was noted. Five of the bacterial counts were over 999 per sq. cm. and yellow micrococci regularly predominated, although various other types of bacteria were also present. The condition of the churns giving these excessive counts indicated that none of them had been treated properly. All showed more or less fat on the wood, four of them showed an accumulation of curd and in one a slight objectionable odor was evident.

Three of the beef infusion agar discs, secured on the churns in commercial use, that illustrate the extreme conditions encountered are shown in figs. 1, 2 and 3.

Yeast and mold counts were made in 19 of the 32 examinations. In all cases the yeast and the mold counts were much

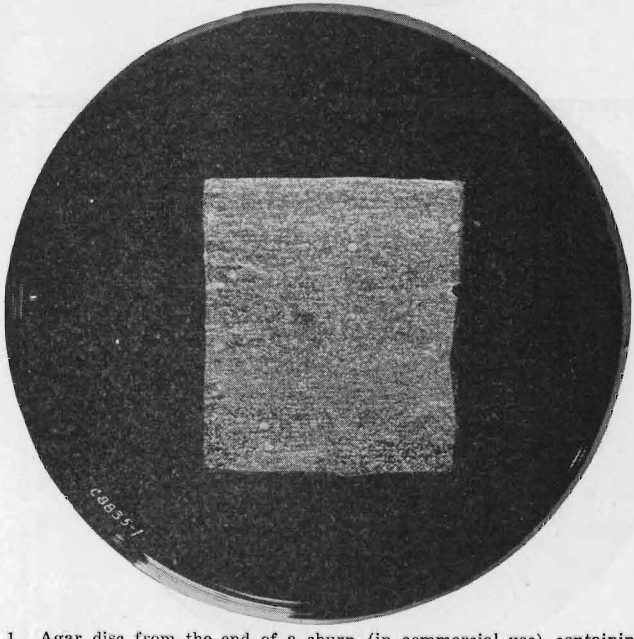

Fig. 1. Agar disc from the end of a churn (in commercial use) containing large numbers of bacteria.

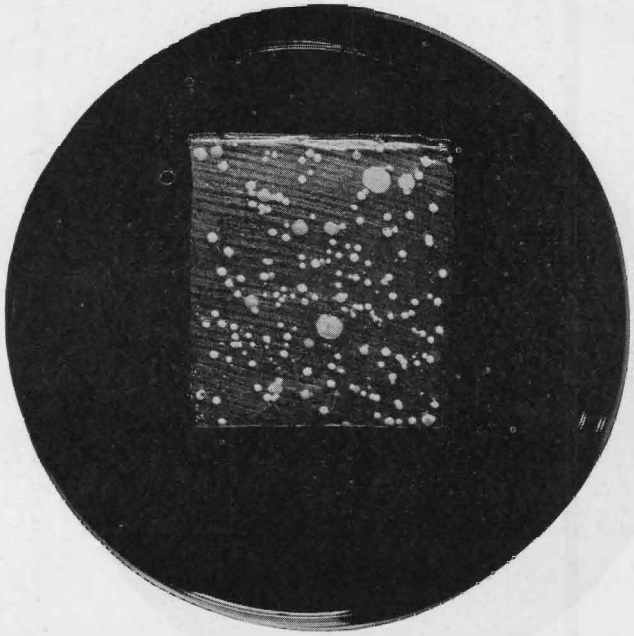

Fig. 2. Agar disc from the end of a churn (in commercial use) containing comparatively few bacteria.

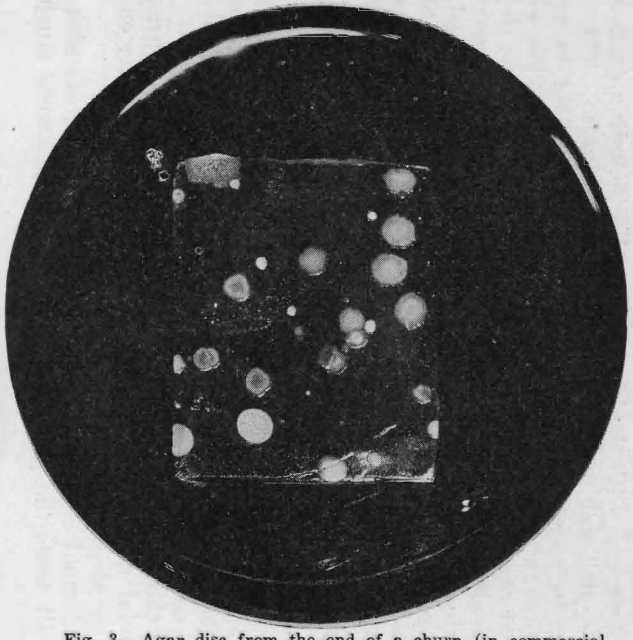

Fig. 3. Agar disc from the end of a churn (in commercial use) containing comparatively few bacteria.

lower than the bacterial counts. The yeast counts varied from 0 to 1.20 per sq. cm. and the mold counts from 0.06 to 5.46 per sq. cm., but yeast and mold counts were not made on a number of the churns heavily contaminated with bacteria. The mold count on a churn was higher than the yeast count in 18 of the 19 comparisons.

At the time the agar discs were prepared on a churn, information on the washing procedure used with it was secured from the plant manager; in general, the temperature of the water employed and the period it was held in the churn were estimated. The washing procedures varied widely and many of them were very evidently inadequate. Washing powder or lime was employed regularly with only a few of the churns, and with some of them these materials were never used. The churns washed without powders usually showed fat on the wood, and in certain of them curd was evident in the angles and crevices. Some of the churns were moist inside, and when curd was present in these it commonly had a pronounced odor; in general, the moist churns had more or less of an objectionable odor. Presumably, the temperature of the final rinse water was too low and the period of exposure too short to secure a satisfactory destruction of organisms or to leave the wood hot enough so that it would dry properly.

The microbiological condition that would be expected in a churn from the washing procedure reported for it frequently was not in agreement with the results of the agar disc counts, due probably to the indefinite statements made when temperatures are never taken nor exposures timed. The general sanitary condition of a plant was usually a good index of the condition of the churn, and it appeared that when a plant was well cared for the churn regularly received the proper attention, while when a plant was poorly cared for the churn was also neglected. In this connection it is of interest to note that in the eight instances in which two churns were examined in a plant or the same churn was examined on two occasions, the results were in general agreement in six instances while in two they were not.

The data secured on the churns in commercial use show that there were wide variations in the microbiological condition of the churns examined.

PART 2. REGULAR TREATMENT OF CHURNS WITH HOT WATER

The efficiency of the regular treatment of churns with hot water was studied on two churns in the Iowa State College butter laboratory by means of agar disc counts.

The general procedure used on the churns was as follows:

1. The milk solids were rinsed from the churn by adding

water at a temperature of from 100° to 120° F., revolving the churn for several minutes and then draining it.

2. The churn was filled one-third to one-half full of water at 170° to 180° F., soda ash added at the rate of about 1 pound per 100 gallons of water, the churn revolved in high gear for about 15 minutes and drained.
3. The churn was filled about one-half full of water at not less than 180° F. and preferably at 200° F. or higher and revolved in high gear for from 15 to 20 minutes. It was then drained thoroughly and turned so that the door opening was about two-thirds of the way up; in this position the churn dried rapidly. After the churn was dry a frame covered with screen was placed in the door opening. In some of the later trials muslin was used over the screen as a protection against contamination from the air. About once a week slaked lime (free from sand, gravel, etc.) was added to the last water at the rate of about 1 pound per 100 gallons of water.
4. Before use the churn was rinsed by filling it one-third to one-half full of water at about 50° F., revolving in high gear for 5 minutes and then draining.

Churn A was a four-roller type of 600 pounds capacity in good mechanical condition. The interior surface was slightly slivered in places but was entirely free from curd and fat. At the beginning of the trials the churn had been in use about 5 years. The agar disc counts obtained on churn A during the period from Jan. 5 to Nov. 25, 1931, are given in table II.

The 53 bacterial counts ranged from 0.4 to 88 per sq. cm. and averaged 17.4. In general, the bacteria were of very few types and were largely members of the genus Bacillus; the higher counts commonly included more types than the lower counts and, in a few instances, included yellow micrococci. The 53 yeast counts varied from 0 to 0.25 per sq. cm. and averaged 0.04, while the 53 mold counts ranged from 0 to 2.56 per sq. cm. and averaged 0.28. In most cases the mold count on churn A was higher than the yeast count, and both the yeast and the mold counts were commonly lower than the bacterial count, the difference usually being comparatively large.

The discs prepared on the ends and the barrel of the churn commonly showed smaller numbers of organisms (bacteria or yeasts or molds) than those prepared on a shelf or a roller. Since the shelf or roller used for the preparation of the discs was one directly exposed to contamination from the air, this distribution suggests that organisms coming from the air during the periods the churn is idle may be a factor in churn contamination.

TABLE II. AGAR DISC COUNTS ON CHURN A AFTER TREATMENT WITH HOT WATER

Date of exam.	Organisms per sq. cm.		
	Bacteria	Yeasts	Molds
1/ 5/31	41	0	0
1/ 7/31	18	0	.04
1/ 8/31	16	0	0
1/ 9/31	7.7	0	.05
1/12/31	32	0	0
1/13/31	20	0	.14
1/15/31	28	.05	.03
1/20/31	3.3	0	.15
1/22/31	1.2	0	.63
1/24/31	2.7	.01	.20
1/26/31	47	0	.06
1/27/31	28	.02	.06
1/28/31	18	0	1.00
1/29/31	19	.20	.05
2/ 5/31	27	.22	.10
2/ 9/31	29	.25	.40
2/10/31	33	.10	.04
2/11/31	33	0	.08
2/14/31	2.3	.03	.08
2/16/31	9.4	.10	0
2/19/31	41	0	0
2/26/31	14	.04	.07
3/19/31	7.5	0	0
3/26/31	57	0	.05
3/28/31	34	.02	.04
3/30 31	32	0	.14
3/31/31	19	0	.02
4/ 2/31	12	.02	.08
4/ 4/31	14	.04	.04
4/ 6/31	20	0	.10
4/ 9/31	27	0	0
4/10/31	14	.04	.07
5/11/31	88	.10	.30
6/ 9/31	20	.04	.56
9/24/31	4.5	0	.73
9/28/31	1.9	0	1.05
9/29/31	1.1	.15	1.10
10/ 1/31	1.4	0	1.60
10/ 2/31	1.3	.15	1.03
10/ 6/31	.8	0	.22
10/ 7/31	5.4	.12	.37
10/ 9/31	2.8	.04	.23
10/12/31	.9	0	.26
10/14/31	.4	0	.36
11/ 2/31	11	0	.28
11/ 3/31	55	0	.25
11/ 4/31	9.8	0	.10
11/10/31	2.4	.10	.13
11/11/31	1.7	0	.42
11/17/31	1.0	.14	2.56
11/18/31	3.8	.03	.04
11/20/31	2.2	.20	.46
11/25/31	1.5	0	.03
Average	17.4	.04	.28

Churn B was a single roller type of 600 pounds capacity in good mechanical condition. The interior surface was rough but was not slivered and was free from curd and fat. It had been in use about 4 years at the beginning of the trials. Table III gives the agar disc counts secured on churn B from Oct. 13, 1930, to April 4, 1932.

TABLE III. AGAR DISC COUNTS ON CHURN B AFTER TREATMENT WITH HOT WATER

Date of exam.	Organisms per sq. cm.		
	Bacteria	Yeasts	Molds
10/13/30	9.0		
10/16/30	24		
10/18/30	18		
10/23/30	43		
11/12/30	23		
11/13/30	20		
11/20/30	10	.21	1.05
12/12/30	9.7	.06	.44
1/ 5/31	18	0	0
1/ 6/31	5.0	0	.06
1/ 8/31	8.0	0	.03
1/ 9/31	13	0	.15
1/12/31	27	.13	1.20
1/26/31	17	0	0
1/27/31	18	.13	.25
1/29/31	20	.15	.83
2/ 3/31	11	.06	.06
2/ 9/31	27	.08	.39
2/10/31	5.3	0	.08
2/11/31	7.7	0	0
2/12/31	2.0	0	.10
2/14/31	13	.03	0
2/19/31	41	0	0
2/21/31	2.8	0	.15
2/23/31	44	0	0
2/25/31	13	.10	.33
2/28/31	3.7	0	.01
3/ 3/31	27	.02	.07
3/ 5/31	9.8	.06	.22
3/ 7/31	13	.06	.30
3/ 9/31	29	.12	.03
3/12/31	28	.08	.14
3/16/31	44	0	.07
3/24/31	9.4	0	.02
3/28/31	19	.03	.04
3/31/31	12	.03	.06
4/ 6/31	15	.01	.04
4/20/31	13	0	.02
4/21/31	2.9	.02	.03
4/24/31	6.8	0	.17
4/28/31	13	0	.06
4/30/31	2.8	0	.46
5/ 4/31	12	.12	.04
5/ 5/31	4.9	0	.07
5/11/31	1.8	.05	.22
5/12/31	5.1	0	.27
5/14/31	6.3	0	.19
5/25/31	110	0	.04
5/28/31	151	.03	.16
5/29/31	86	0	.37
6/ 1/31	18	.05	.71
6/ 3/31	66	0	.02
6/ 4/31	29	.05	1.04
6/ 5/31	3.8	0	.13
6/ 9/31	5.8	.13	.60
6/10/31	13	.03	.14
6/11/31	7.6	0	.10
6/12/31	2.1	.04	.14
6/18/31	5.2	0	.10
6/24/31	6.0	0	.15
6/25/31	23	0	.13
6/26/31	10	0	.09
6/27/31	29	0	.10
7/17/31	16	0	.10
9/23/31	4.9	0	.86

TABLE III—(Continued)

Date of exam.	Organisms per sq. cm.		
	Bacteria	Yeasts	Molds
9/29/31	1.4	.07	.69
10/ 5/31	2.5	.04	1.04
10/ 6/31	1.1	.08	.28
10/ 7/31	1.0	.10	.32
10/ 9/31	.5	.04	.26
10/12/31	.3	0	.27
10/20/31	1.0	.06	.28
10/21/31	.5	0	1.17
10/27/31	21	.04	1.02
11/ 2/31	685	0	.40
11/ 4/31	10	.10	.16
11/10/31	14	.03	.08
11/11/31	1.3	0	.05
11/12/31	2.3	.16	.18
11/17/31	1.5	.03	.19
11/18/31	1.4	.03	.19
11/20/31	1.6	.05	.44
11/24/31	2.3	0	.01
11/26/31	1.0	.03	.12
12/ 7/31	.6	0	.07
12/ 9/31	1.1	0	.26
12/11/31	1.7	0	.01
12/15/31	.6	.01	.38
1/ 6/32	2.3	0	.23
1/21/32	1.0	0	.14
3/ 9/32	2.2	.04	.09
4/ 4/32	9.8	.07	.39
Average	22.5	.03	.24

The 92 bacterial counts varied from 0.3 to 685 per sq. cm. and averaged 22.5; if the highest count, which was excessive for no apparent reason, is excluded the average is 15.2. The types of bacteria present were essentially the same as those in churn A. The 86 yeast counts ranged from 0 to 0.21 per sq. cm. and averaged 0.03 and the 86 mold counts varied from 0 to 1.20 per sq. cm. and averaged 0.24. The mold count on churn B was nearly always higher than the yeast count, and both the yeast and the mold counts were usually much lower than the bacterial count. As with churn A, smaller numbers of organisms were found on the ends and the barrel than on a shelf.

The predominance of bacteria belonging to the genus Bacillus in beef infusion agar discs prepared on churns regularly treated with hot water is illustrated in fig. 4.

The counts on both churn A and churn B show no evidence of a seasonal variation in the numbers of organisms present. Early in the periods during which the churns were examined, bacterial counts of less than 10 per sq. cm. were not as numerous as late in these periods; this relationship may have been influenced by the desire of the churn operators, who knew that the churns were being examined microbiologically, to clean the churns as thoroughly as possible with the method in use. The churns were consistently free from curd, fat and objectionable odors.

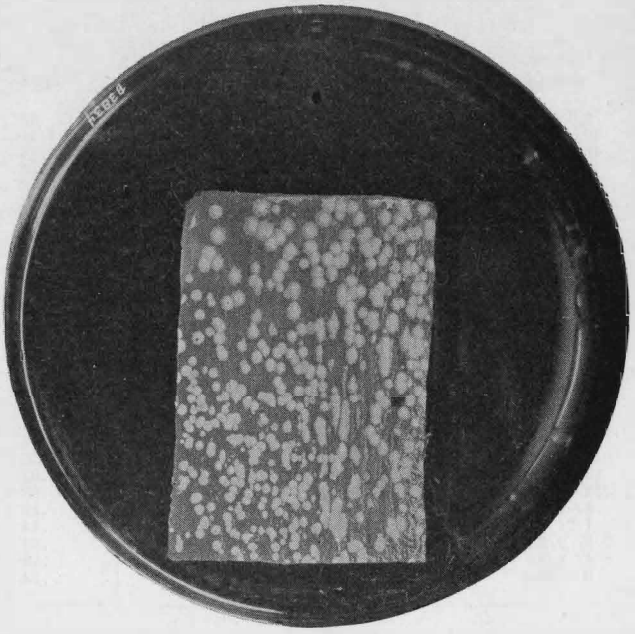

Fig. 4. Agar disc from the end of a churn regularly treated with hot water; the organisms are almost entirely Bacillus types.

The data secured indicate that the churns treated regularly with hot water commonly contained comparatively small numbers of organisms, as determined by the agar disc method, although there was some variation in the numbers present. The bacteria found in the churns were largely types resistant to heat.

PART 3. USE OF CHEMICALS ON CHURNS TREATED WITH HOT WATER

The agar disc counts on the churns carefully treated with hot water showed that although the counts were commonly comparatively low a number of organisms, particularly spore-forming bacteria, were still present. Trials were, accordingly, carried out in which an attempt was made to reduce the numbers of organisms in churns treated with hot water through the action of various chemicals.

(a) USE OF CHLORINE COMPOUNDS ON CHURNS TREATED WITH HOT WATER

The extensive use of chlorine compounds for the destruction of organisms in various types of equipment employed in han-

dling dairy products has led to their use in the treatment of churns. The data presented in Part 1 on the extent of contamination of churns in commercial use include counts on five chlorine-treated churns. Three of these churns had very low bacterial counts while the other two had high counts. Of the three churns with very low counts, one (J) was treated with sodium hypochlorite after each washing and the other two (Aa 4/13/31 and Ab) received weekly treatments with this compound. Of the two churns with high counts, one (N) was treated twice a week with a sodium hypochlorite trisodium phosphate compound and the other (L 3/2/31) was treated every 2 weeks with sodium hypochlorite. According to calculations based on the amounts of the chemicals reported as being employed, the solutions used on the churns contained from about 10 to about 125 ppm. available chlorine.

The chlorine compounds used on churns treated with hot water were sodium hypochlorite, chlorinated lime and calcium hypochlorite. The sodium hypochlorite was prepared according to the method outlined by Zoller (25) and the stock solution contained about 3 percent of available chlorine. The chlorinated lime was purchased in sealed 1-pound cans and, according to the label, contained over 30 percent of available chlorine. A calcium hypochlorite stock solution was prepared from a powdered commercial product labeled as containing 65 percent of available chlorine; the solution contained about 2.5 percent available chlorine.

The method used for studying the effect of the chlorine was as follows: Agar discs were prepared on churns that had dried thoroughly following hot water treatment. Ten gallons of tap water, to which had been added a quantity of a chlorine compound calculated to give the desired concentration of available chlorine, were added to the churn, the temperature taken and the churn revolved in high gear for the desired time; in some of the trials the temperatures after exposure were also taken. Samples of the rinse water for chlorine determinations were taken before and after exposure to the churn. After the treated churn had been drained and allowed to dry, agar discs were again prepared, using sterile litmus milk to moisten the surfaces examined in order to eliminate the effect of residual chlorine. Churns A and B (see Part 2) were used. The results obtained are presented in table IV.

In the 11 trials with sodium hypochlorite the concentrations of available chlorine in the solutions used ranged from 56 to 102 ppm. before exposure to the churn and from 5 to 30 ppm. (10 trials only) after exposure. The initial temperatures of the solutions varied from 70° to 122° F. and the periods of exposure from 15 to 45 minutes. According to the agar disc counts the

TABLE IV. USE OF CHLORINE COMPOUNDS ON CHURNS TREATED WITH HOT WATER.

Date	Chlorine solution						Agar disc counts before chlorine treatment			Agar disc counts after chlorine treatment		
	Before exposure		Period of exposure min.	After exposure		Bacteria per ml.	Organisms per sq. cm.			Organisms per sq. cm.		
	Avail. chlorine ppm.	Temp. °F.		Avail. chlorine ppm.	Temp. °F.		Bacteria	Yeasts	Molds	Bacteria	Yeasts	Molds
Sodium hypochlorite												
2/19/31	58	120	15	14	.....	72	41	0	0	.6	0	0
2/21/31	60	122	15	20	.....	10	2.8	0	.15	.2	0	0
2/25/31	56	118	15	16	.....	22	13	.10	.30	.5	0	.03
2/26/31	63	76	15	.....	.....	101	14	.04	.07	1.2	0	.02
2/28/31	63	70	15	12	.....	25	3.7	0	.01	<.1	0	0
3/ 3/31	60	120	15	9	.....	30	27	.02	.07	.3	0	.04
3/ 5/31	60	120	45	5	.....	49	9.8	.06	.20	1.3	0	.10
3/ 7/31	60	70	15	15	.....	20	13	.06	.30	2.4	.01	.03
3/ 9/31	98	70	30	30	.....	10	29	.12	.03	.4	0	.12
3/12/31	102	115	15	22	.....	7	28	.08	.14	1.7	.02	.02
3/16/31	96	120	15	24	.....	20	44	0	.07	.3	0	0
					Average	33.3	20.5	.04	.12	.8	<.01	.03
Chlorinated lime												
4/20/31	102	108	15	23	.....	.....	13	0	.02	<.1	0	.01
4/21/31	105	115	5	30	.....	.....	2.9	.02	.03	<.1	0	.03
4/24/31	104	115	15	12	.....	.....	6.8	0	.17	.4	0	.03
4/28/31	88	110	20	8	.....	.....	13	0	.06	.2	0	.09
4/30/31	97	108	10	28	.....	.....	2.8	0	.46	.1	0	.10
5/ 4/31	97	110	15	10	.....	.....	12	.03	.14	.3	0	.04
5/ 5/31	100	80	5	42	.....	.....	4.9	0	.07	.2	0	.07
5/11/31	98	110	15	15	.....	.....	1.8	.01	.22	<.1	0	.03
5/12/31	104	112	10	27	.....	.....	5.1	0	.27	.1	0	.09
5/14/31	104	114	23	11	.....	.....	6.3	0	.19	.1	0	.12
5/25/31	98	139	20	6	.....	.....	110	0	.04	.8	0	.02
					Average	.....	16.2	.01	.15	.2	0	.06

TABLE IV—(Continued)

Calcium hypochlorite

5/28/31	104	122	18	7	92	.....	151	.03	.16	.1	0	.12
5/29/31	99	124	18	9	95	.....	86	0	.37	.1	0	.12
6/ 1/31	110	120	15	9	93	.....	18	.05	.71	.4	0	.20
6/ 3/31	100	80	15	10	.....	.....	66	0	.02	<.1	0	.07
6/ 4/31	105	122	15	14	95	.....	29	.05	1.20	.1	0	.13
6/ 5/31	95	121	16	12	90	.....	3.8	0	.13	0	0	.05
6/ 9/31	105	122	18	6	104	.....	5.8	.05	.60	.2	.01	.34
6/10/31	113	135	13	11	106	.....	13	.03	.14	.2	0	.09
6/11/31	100	121	18	6	104	.....	7.6	0	.10	.2	.03	.35
6/12/31	122	125	17	13	98	.....	2.1	.04	.14	<.1	0	.07
6/18/31	115	118	18	9	96	.....	5.2	0	.10	.8	0	.05
					Average	.....	35.2	.02	.33	.2	<.01	.14

bacteria before treatment of the churns ranged from 2.8 to 44 per sq. cm. and averaged 20.5, while after treatment they ranged from less than 0.1 to 2.4 per sq. cm. and averaged 0.8; the yeasts before treatment varied from 0 to 0.12 per sq. cm. and averaged 0.04, while after treatment no yeasts were detected in nine of the trials and in the other two the counts were 0.01 and 0.02 per sq. cm.; the molds before treatment ranged from 0 to 0.30 per sq. cm. and averaged 0.12, and after treatment they varied from 0 to 0.12 and averaged 0.03. The bacteria in the chlorine solutions after exposure to the churn varied from 7 to 101 per ml. and averaged 33.3.

In the 11 trials with chlorinated lime the concentrations of available chlorine in the solutions employed ranged from 88 to 105 ppm. before exposure to the churn and from 6 to 42 ppm. after exposure. The initial temperatures of the solutions varied from 80° to 139° F. and the periods of exposure from 5 to 23 minutes. With the agar disc method the bacterial counts before treatment of the churns ranged from 1.8 to 110 per sq. cm. and averaged 16.2, while after treatment they ranged from less than 0.1 to 0.8 per sq. cm. and averaged 0.2; before treatment yeasts were detected in only 3 of the 11 trials, the counts being 0.02, 0.03 and 0.01 per sq. cm., while after treatment yeasts were not detected in any of the trials; the mold counts before treatment varied from 0.02 to 0.46 per sq. cm. and averaged 0.15, while after treatment they ranged from 0.01 to 0.12 per sq. cm. and averaged 0.06.

In the 11 trials with calcium hypochlorite the concentrations of available chlorine in the solutions used ranged from 95 to 122 ppm. before exposure to the churn and from 6 to 14 ppm. after exposure. The initial temperatures of the solutions varied from 80° to 135° F., the periods of exposure from 13 to 18 minutes and the temperatures after exposure from 90° to 106° F. (10 trials only).

According to the agar disc counts the bacteria before treatment of the churns varied from 2.1 to 151 per sq. cm. and averaged 35.2, and after treatment they varied from 0 to 0.8 per sq. cm. and averaged 0.2; the yeast counts before treatment ranged from 0 to 0.05 per sq. cm. and averaged 0.02, while after treatment yeasts were detected in only two trials, the counts being 0.01 and 0.03 per sq. cm.; the mold counts before treatment ranged from 0.02 to 1.20 per sq. cm. and averaged 0.33, while after treatment they varied from 0.05 to 0.35 per sq. cm. and averaged 0.14.

No definite correlation between the initial temperature or concentration of the solution or the period of exposure and the destruction of organisms is evident in the data secured, due

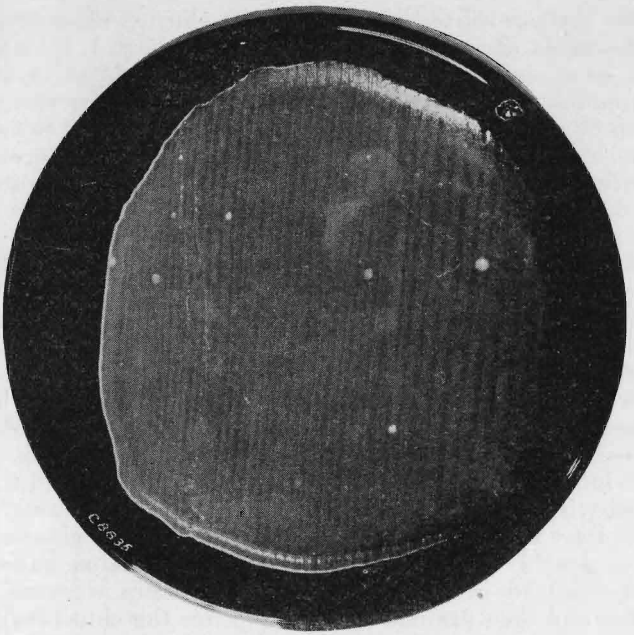

Fig. 5. Agar disc from the shelf of a churn treated with sodium hypochlorite following treatment with hot water.

presumably to the comparatively small numbers of organisms originally present and to the unusually small numbers present after each of the chlorine treatments. Marked decreases in available chlorine during exposure of the solutions to the churn occurred with all three compounds; there was no close correlation between the extent of the decrease and the temperature or period of exposure of the solution, although commonly large decreases occurred with high temperatures or long exposures.

In general the data show that the use of chlorine on the churns treated with hot water resulted in reductions in the numbers of organisms, as determined by agar disc counts. The reductions were especially pronounced with the bacteria. Figure 5 shows an agar disc from the shelf of a churn treated with sodium hypochlorite following treatment with hot water; only a very few colonies are present.

(b) USE OF SODIUM CHLORIDE ON CHURNS TREATED WITH HOT WATER

The restraining action of sodium chloride on many of the organisms in salted butter suggests its use in high concentra-

tion for the control of the microflora of churns. The counts on the churns in commercial use, reported in Part 1, include one count on a churn treated with sodium chloride (C 4/13/31). The treatment involved rinsing out the churn with warm water, adding 80 gallons of water and 40 pounds of butter salt to the churn, heating the solution to nearly boiling with steam and revolving the churn in high gear for 5 minutes; the churn was then drained and allowed to dry. After the drying, the entire inner surface of the churn was covered with fine crystals. The counts on the treated churn showed 36 bacteria, 0.13 yeasts and 0.66 molds per sq. cm. The same churn had been examined about 5 months earlier, when the sodium chloride treatment was not being used, and the count was 228 bacteria per sq. cm.; acidified whey agar discs were not prepared but the discs for bacterial counts showed 4.9 molds per sq. cm. It should be recognized that the temperature of the salt solution used was undoubtedly a factor in reducing the number of organisms.

The influence of sodium chloride on the organisms in a churn treated with hot water was studied in seven trials using churn A (see Part 2). Agar discs were prepared on the churn, 3 gallons of a cold, saturated sodium chloride solution added, the churn revolved in high gear with the rollers working for 5 minutes and then drained and dried; after the churn had stood for from 6 to 48 hours agar discs were again prepared. The inner surface of the churn treated with sodium chloride showed a covering of fine salt crystals. This salt probably had no appreciable effect on the agar disc counts since, when a churn was rinsed with water and the water plated, the addition of 1 ml. of saturated sodium chloride solution to some of the plates did not influence the counts. Table V presents the results obtained with the sodium chloride treatment.

The bacterial counts on the churn before the salt treatment ranged from 14 to 57 per sq. cm. and averaged 28.1, while

TABLE V. USE OF SODIUM CHLORIDE ON CHURNS TREATED WITH HOT WATER

Date	Agar disc counts on churns treated with hot water				Agar disc counts on churns after use of sodium chloride			
	Hours since treatment	Organisms per sq. cm.			Hours since treatment	Organisms per sq. cm.		
		Bacteria	Yeasts	Molds		Bacteria	Yeasts	Molds
3/26/31	6	57	0	.08	6	41	.04	.03
3/28/31	4	34	.05	.04	48	30	.08	.22
3/30/31	4	32	0	.14	16	37	0	.08
3/31/31	4	19	0	.02	16	33	0	.19
4/ 4/31	6	14	.04	.04	48	11	.03	.22
4/ 9/31	4	27	0	0	16	20	.03	.28
4/10/31	4	14	.05	.07	16	18	.05	.11
	Average	28.1	.02	.06		27.1	.03	.16

those on the churn after the treatment ranged from 11 to 41 per sq. cm. and averaged 27.1. The yeast counts before the treatment varied from 0 to 0.05 per sq. cm. and averaged 0.02, while those after the treatment varied from 0 to 0.08 per sq. cm. and averaged 0.03. The mold counts before the treatment ranged from 0 to 0.14 per sq. cm. and averaged 0.06, while after the treatment they varied from 0.03 to 0.28 per sq. cm. and averaged 0.16.

In general the use of a cold, saturated sodium chloride solution on a churn that had been treated with hot water did not significantly influence the numbers of organisms present, as determined by agar disc counts. Air contamination undoubtedly added considerable numbers of organisms to the churn after treatment since it stood for from 6 to 48 hours before the counts were made.

PART 4. TREATMENT OF HIGHLY CONTAMINATED CHURNS

A churn that is given proper care each time it is used, so that excessive numbers of organisms are never present in it, undoubtedly presents a different problem from one containing an enormous number of organisms and, accordingly, trials were carried out on highly contaminated churns.

Two churns were employed in the studies. Churn C was an experimental churn of 70 pounds capacity, in good mechanical condition, that had been in use about 2 years. It was heavily contaminated before each trial by washing carelessly, after agitating about a gallon of buttermilk in it for several minutes, and repeating this process two or three times at intervals of 2 or 3 days. Churn F, which had a capacity of 300 pounds, was in rather poor mechanical condition and the wood was somewhat spongy and slightly rough. It was about 12 years old but had not been used for about 1 year. One month previous to its use in the trials it had been washed and poorly drained and, as a result, the interior developed a profuse mold growth; the growth in a part of one end of the churn is shown in fig. 6. The churn was studied only at rather long intervals, so that with the careless washing and drying it regained a heavy contamination between trials.

(a) TREATMENT OF HIGHLY CONTAMINATED CHURNS WITH HOT WATER

The procedure used in studying the hot water treatment of highly contaminated churns was as follows: Counts were made on the churn by the agar disc and rinse methods. The water used with the rinse method was left in the churn, hot water added until the churn was as full as desired and the total quantity of water heated to the desired temperature with steam.

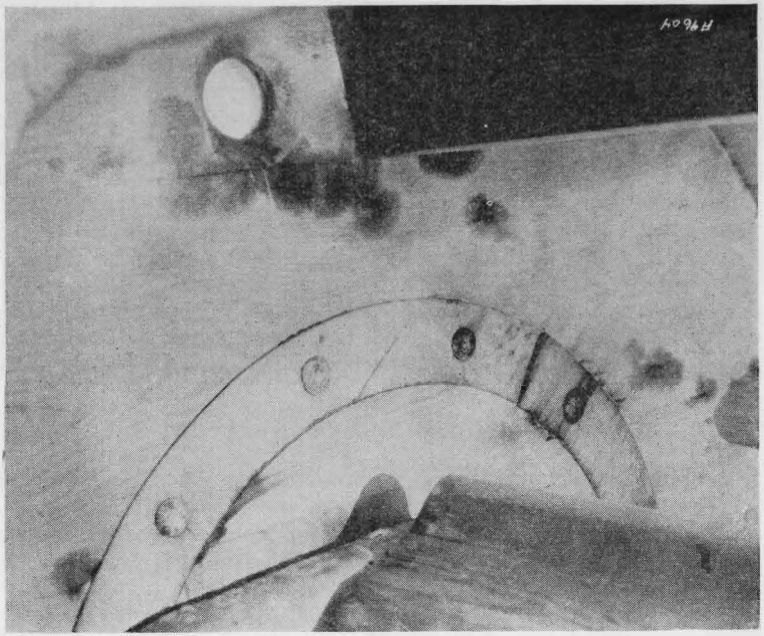

Fig. 6. Mold growth in part of one end of churn F before the churn was used in the trials on the treatment of highly contaminated churns.

The churn was revolved for the desired period, the temperature of the water taken and also a sample of the water for plating. The churn was then drained, the rinse method again applied and, after draining and drying, agar discs were again prepared, usually within 4 hours. The results obtained in seven trials are shown in table VI.

The churns were filled from one-half full to full with water at temperatures ranging from 180° to 208° F. The periods of exposure varied from 10 to 70 minutes and the temperatures after exposure from 168° to 196° F. The bacterial counts on the hot water after exposure to the churns ranged from 5,000 to 23,000 per ml. and averaged 11,440; no yeasts or molds were ever detected in this water. The bacterial counts by the rinse method before treatment ranged from 1,000,000 to 13,300,000 per ml. and averaged 3,624,000, while after treatment they ranged from 2,800 to 30,000 per ml. and averaged 12,480; compared with these values the numbers of organisms in the water used for rinsing the churns are negligible. The yeast counts before treatment varied from 6 to 950 per ml. and averaged 209, while after treatment they varied from 0 to 42 per ml. and averaged 7. The mold counts before treatment ranged from 23 to 830,000

TABLE VI. TREATMENT OF HIGHLY CONTAMINATED CHURNS WITH HOT WATER

Date	Churn	Hot water used for treatment					Plate counts of rinse water before hot water treatment				Plate counts of rinse water after hot water treatment				Agar disc counts before hot water treatment			Agar disc counts after hot water treatment		
		Fullness of churn	Temp. before exposure °F.	Period of exposure (min.)	Temp. after exposure °F.	Bacteria per ml. after exposure	Water used for rinse; bacteria per ml.	Water after exposure to churn Organisms per ml.			Water used for rinse; bacteria per ml.	Water after exposure to churn Organisms per ml.			Organisms per sq. cm.			Organisms per sq. cm.		
								Bacteria	Yeasts	Molds		Bacteria	Yeasts	Molds	Bacteria	Yeasts	Molds	Bacteria	Yeasts	Molds
2/26/32	C	2/3	199	10	190	5,000	11	13,300,000	300	1,450	33	16,000	42	76	>2,000	26.60	2.03	106	.07	.18
3/ 5/32	C	2/3	204	20	192	7,500	151	5,250,000	45	150	43	11,200	2	16	>1,500	.43	.23	165	.01	.12
3/11/32	C	2/3	208	30	196	7,800	38	2,300,000	60	35	71	2,800	0	1	>1,000	.28	.39	54	.04	.12
3/24/32	C	full	204	40	194	10,800	3	1,100,000	52	23	2	5,300	1	1	400	.20	.10	18	0	0
3/31/32	C	full	207	60	191	13,800	18	1,100,000	950	290	40	3,550	0	1	600	.10	.27	16	0	.02
4/22/32	F	1/2	208	70	196	23,000	0	1,000,000	50	830,000	5	18,500	0	8	800	Discs covered with molds		12	.02	1.12
5/ 3/32	F	1/2	180	60	168	12,200	82	1,320,000	6	150	6	30,000	1	2	82	.01	.74	16	0	.01
Average						11,440	43	3,624,000	209	118,900	29	12,480	7	15	912	4.60	.63	55	.02	.22

per ml. and averaged 118,900, the very high average being due primarily to one excessive count (830,000 per ml.) on a badly molded churn; after treatment the mold counts ranged from 1 to 76 per ml. and averaged 15.

With the agar disc method, the bacterial counts before treatment ranged from 82 to more than 2,000 per sq. cm. and averaged 912. After treatment the bacterial counts ranged from 12 to 165 per sq cm. and averaged 55. In one trial (churn F 4/22/32) yeast and mold counts were not obtained because the whey agar discs were completely overgrown with molds in 2 days; in the other six trials the yeast counts before treatment varied from 0.01 to 26.60 per sq. cm. and averaged 4.60, while after treatment the seven counts varied from 0 to 0.07 per sq. cm. and averaged 0.02. In the six trials the mold counts before treatment ranged from 0.10 to 2.03 per sq. cm. and averaged 0.63, while after the treatment in the seven trials they ranged from 0 to 1.12 per sq. cm. and averaged 0.22.

In the various trials there is a lack of correlation between the results of the rinse method and those of the agar disc method that is very conspicuous. Hammer and Olson (7) have pointed out that a close relationship between the results of the two procedures would not be expected if what is being measured by each is considered.

Both the plates poured with the rinse water and the agar discs showed that the bacteria in the churns before treatment usually included a variety of types, which were largely micrococci and non-spore forming rods, while those in the churns after treatment were largely Bacillus types.

Taking into account the fullness of the churn in the hot water treatment and the numbers of organisms originally present in the different trials, the data on churn C suggest that as the period of exposure increased there was a tendency for the number of organisms per milliliter of the hot water used in the treatment of the churns to increase. This may have been due to the continual dislodging of organisms during the period of exposure. With churn C also the shorter exposures left comparatively large numbers of organisms in the churn; it should be noted, however, that with the shorter exposures the numbers of organisms originally present in the churn were unusually high. The results obtained with churn F on 4/22/32 are of special interest because of the great destruction of molds secured with the hot water treatment of the churn showing a heavy mold growth.

The hot water treatment of highly contaminated churns effected striking reductions in the numbers of organisms present as determined by the rinse or agar disc method. The heat re-

sistance of certain of the bacteria present in the churns is shown by the survival of considerable numbers of organisms in the hot water used to treat the churns.

(b) TREATMENT OF HIGHLY CONTAMINATED CHURNS WITH CHLORINE COMPOUNDS

Two chlorine compounds, sodium hypochlorite and a chloramine preparation, were used in the treatment of highly contaminated churns. The sodium hypochlorite stock solution was prepared from powdered calcium hypochlorite, sodium carbonate and water and contained about 2.5 percent available chlorine. The chloramine compound was a washing and sterilizing powder containing, according to the label, over 4 percent available chlorine.

The efficiency of the treatment of highly contaminated churns with chlorine was studied by making counts on the churns by both the agar disc and rinse methods before and after treatment. In the rinse method the water was usually heated with steam before exposure so that after exposure the temperature would be approximately that desired, but in no case was it heated to over 125° F. The water was heated in an attempt to measure the action of the chlorine solution largely independent of the temperature at which it was used. After exposure the 10 gallons of water were left in the churn, in order to eliminate the mechanical removal of microorganisms by it, more water was added to give the desired fullness, the calculated amount of chlorine solution or compound was then added and, if necessary, the total heated with steam to the desired temperature. The available chlorine of the solution in the churn before exposure was approximated by adding to 10 gallons of water an amount of the chlorine solution or compound equal to that used in the churn and determining the available chlorine content.

SODIUM HYPOCHLORITE

The results obtained in the nine trials in which a highly contaminated churn was treated with a sodium hypochlorite solution are given in table VII; the churn was filled three-fourths full of the solution in one trial and one-third full in each of the others.

In the eight trials on churn C the concentrations of available chlorine of the solutions used ranged from 75 to 141 ppm., the temperatures from 70° to 142° F. and the periods of exposure from 10 to 60 minutes. After exposure the concentrations of available chlorine in the solutions ranged from 16 to 50 ppm. and the temperatures from 72° to 133° F. The bacterial counts of the solutions after exposure varied from 2 to 550 per ml. and

TABLE VII. TREATMENT OF HIGHLY CONTAMINATED CHURNS WITH SODIUM HYPOCHLORITE

Date	Churn	Chlorine solution: Before exposure: Avail. chlorine ppm.	Before exposure: Temp. °F.	Period of exposure min.	After exposure: Avail. chlorine ppm.	After exposure: Temp. °F.	After exposure: Bacteria per ml.	Plate counts of rinse water before chlorine treatment: Water used for rinse: Bacteria per ml.	Water after exposure to churn: Organisms per ml.: Bacteria	Yeasts	Molds	Plate counts of rinse water after chlorine treatment: Water used for rinse: Bacteria per ml.	Water after exposure to churn: Organisms per ml.: Bacteria	Yeasts	Molds	Agar disc counts before chlorine treatment: Organisms per sq. cm.: Bacteria	Yeasts	Molds	Agar disc counts after chlorine treatment: Organisms per sq. cm.: Bacteria	Yeasts	Molds
1/ 8/32	C	93	70	10	45	72	550	11	6,200,000	94	10	18	850,000	9	45	>2,000	43.36	.64	500	.20	.03
1/14/32	C	104	110	10	50	106	260	30	1,030,000	77	30	10	1,600	1	14	640	.05	.19	20	0	0
1/22/32	C	112	113	10	50	109	20	0	14,600,000	180	10	3	4,900	1	1	>2,000	4.18	.17	86	0	0
1/28/32	C	141	122	30	19	112	60	2	5,700,000	550	70	24	175,000	67	10	>2,000	6.13	.61	13	.06	.02
2/ 5/32	C	133	125	30	26	115	40	10	2,540,000	430	160	10	35,000	51	19	>1,700	6.94	.29	2.3	0	.05
2/11/32	C	105	117	30	16	98	120	3	8,500,000	470	1,020	22	119,000	146	60	>1,500	5.32	1.40	4.7	0	.08
2/18/32	C	130	121	30	39	110	60	1	1,360,000	255	125	16	64,000	0	0	>1,500	.30	.32	9.4	.01	.07
3/18/32	C	75	142	60	18	133	20	2	3,300,000	570	55	10	12,000	0	0	>2,000	0	.24	7.7	0	.03
3/14/32	F	126	128	30	32	113	2	14	3,700,000	20,000	13,000	17	20,000	0	0	>2,000	Too numerous to count		144	0	.05
Average of 9 trials							126	8	5,214,000	2,514	1,609	14	142,400	31	17	1,705	.....	.....	87	.03	.04
Average of 8 trials with churn C							141	7	5,404,000	328	185	14	157,700	34	19	1,668	8.23	.48	80	.03	.04

averaged 141; the yeast and the mold counts were 0 in every trial. The bacterial counts by the rinse method before treatment ranged from 1,030,000 to 14,600,000 per ml. and averaged 5,404,000, while after treatment they ranged from 1,600 to 850,000 per ml. and averaged 157,700. The yeast counts before treatment varied from 77 to 570 per ml. and averaged 328, while after treatment they varied from 0 to 146 per ml. and averaged 34. The mold counts before treatment ranged from 10 to 1,020 per ml. and averaged 185, while after treatment they ranged from 0 to 60 per ml. and averaged 19.

According to the agar disc method the bacteria before treatment ranged from 640 to more than 2,000 per sq. cm. and averaged 1,668, while after treatment they ranged from 2.3 to 500 per sq. cm. and averaged 80. The yeast counts before treatment varied from 0 to 43.36 per sq. cm. and averaged 8.23, while after treatment the counts were 0 in five trials and in the other three they were 0.20, 0.06 and 0.01 per sq. cm. Before treatment the mold counts ranged from 0.17 to 1.40 per sq. cm. and averaged 0.48 while after treatment they ranged from 0 to 0.08 per sq. cm. and averaged 0.04.

In the one trial with churn F a solution containing 126 ppm. available chlorine and at a temperature of 128° F. was exposed to the churn for 30 minutes. After exposure the concentration of available chlorine was 32 ppm., the temperature was 113° F. and the count on the solution was 2 bacteria per ml.; yeasts and molds were not found. The counts by the rinse method before treatment showed 3,700,000 bacteria, 20,000 yeasts and 13,000 molds per ml., while after treatment the counts were 20,000 bacteria, 0 yeasts and 0 molds per ml. The agar discs prepared before treatment were completely overgrown with molds in 2 days, but after incubation for 1 day the bacteria were estimated at more than 2,000 per sq. cm. The agar discs prepared after treatment showed 144 bacteria, 0 yeasts and 0.05 molds per sq. cm. It should be noted that in churn F the high contamination was built up by washing and draining carelessly and then holding for a considerable period.

In the data reported in table VII the lack of correlation between the results of the rinse method and those of the agar disc method is especially conspicuous.

As shown by both the plates poured in the rinse method and the agar discs, the bacteria in the highly contaminated churns were of a variety of types, with micrococci and non-spore forming rods predominant, while those in the treated churns were largely Bacillus types.

The data show no close relationship between the concentration of available chlorine or the temperature or period of exposure or the decrease in available chlorine and the organisms

present in the churns after treatment; however, in the one trial in which an initial temperature below 100° F. was used, the survival of organisms was especially striking.

The treatment of highly contaminated churns with sodium hypochlorite resulted in large reductions in the numbers of organisms present, according to either the rinse or the agar disc method. The general action of sodium hypochlorite on the organisms in churns is shown by the comparatively small numbers of organisms in the solutions after exposure to the churns.

CHLORAMINE PREPARATION

The results of five trials in which a highly contaminated churn (churn C in all cases) was treated with a commercial chloramine preparation are shown in table VIII.

The concentrations of available chlorine of the solutions used ranged from 28 to 113 ppm., the temperatures from 135° to 192° F. and the periods of exposure from 20 to 80 minutes; after exposure the concentrations of available chlorine of the solutions ranged from 18 to 95 ppm., and the temperatures from 128° to 176° F. The bacterial counts on the solutions after exposure (four trials) varied from 7,300 to 15,300 per ml. and averaged 10,400, while yeasts and molds were never found. With the rinse method (four trials) the bacterial counts before treatment ranged from 274,000 to 7,300,000 per ml. and averaged 2,611,000, while after treatment they ranged from 8,000 to 29,000 per ml. and averaged 13,800; the yeast counts before treatment varied from 400 to 2,190 per ml. and averaged 963, while after treatment they ranged from 0 to 21 per ml. and averaged 7; the mold counts before treatment ranged from 55 to 130 per ml. and averaged 100, while after treatment they varied from 2 to 10 per ml. and averaged 5.

According to the agar disc method the bacterial counts before treatment ranged from 41 to more than 2,000 per sq. cm. and averaged 870, while after treatment they ranged from 18 to 103 per sq. cm. and averaged 47; the yeast counts before treatment varied from 0 to 1.80 per sq. cm. and averaged 0.78, while after treatment they varied from 0 to 0.08 per sq. cm. and averaged 0.03; the mold counts before treatment ranged from 0.07 to 1.70 per sq. cm. and averaged 0.82, while after treatment they ranged from 0 to 2.70 per sq. cm. and averaged 0.58.

The types of bacteria found before and after treatment with chloramine solutions were essentially the same as with the churns on which sodium hypochlorite solutions were used.

Compared with the sodium hypochlorite solutions, the decreases in the available chlorine in the chloramine solutions during the treatment of churns were small, especially when the temperatures at which the chloramine solution was used is con-

TABLE VIII. TREATMENT OF A HIGHLY CONTAMINATED CHURN WITH A CHLORAMINE PREPARATION

Date	Chlorine solution: Before exposure: Avail. chlorine ppm.	Chlorine solution: Before exposure: Temp. °F.	Chlorine solution: Period of exposure min.	Chlorine solution: After exposure: Avail. chlorine ppm.	Chlorine solution: After exposure: Temp. °F.	Chlorine solution: After exposure: Bacteria per ml.	Plate counts of rinse water before chlorine treatment: Water used for rinse Bacteria per ml.	Plate counts of rinse water before chlorine treatment: Water after exposure to churn Organisms per ml.: Bacteria	Yeasts	Molds	Plate counts of rinse water after chlorine treatment: Water used for rinse Bacteria per ml.	Plate counts of rinse water after chlorine treatment: Water after exposure to churn Organisms per ml.: Bacteria	Yeasts	Molds	Agar disc counts before chlorine treatment: Organisms per sq. cm.: Bacteria	Yeasts	Molds	Agar disc counts after chlorine treatment: Organisms per sq. cm.: Bacteria	Yeasts	Molds
4/ 7/32	113	192	20	91	176	9,200	8	7,300,000	400	95	1	29,000	2	2	>2,000	.78	.07	103	0	.02
4/14/32	101	190	60	88	165	.....	.....	.....	.....	.....	.....	.....	.....	.....	125	1.10	.74	18	0	0
4/22/32	93	188	80	84	158	9,800	0	2,170,000	770	120	5	8,000	0	5	>2,000	1.80	1.70	32	.05	2.70
4/29/32	102	135	30	95	128	7,300	1	700,000	2,190	55	16	8,200	21	10	186	.20	1.10	55	.08	.10
5/ 3/32	28	190	60	18	168	15,300	80	274,000	490	130	6	10,000	3	3	41	0	.48	27	.02	.06
Average						10,400	22	2,611,000	963	100	7	13,800	7	5	870	.78	.82	47	.03	.58

sidered. In general, the numbers of organisms per milliliter of the chloramine solutions after exposure were larger than the numbers per milliliter of the sodium hypochlorite solutions.

The treatment of highly contaminated churns with chloramine resulted in pronounced reductions in the numbers of organisms present, as determined by either the rinse or agar disc method.

PART 5. CONTAMINATION OF CHURNS FROM THE AIR

The agar disc counts on the ends and barrels of the churns treated with hot water were commonly lower than those on the shelves and rollers which were more directly exposed to the organisms falling from the air (see Part 2), and this relationship suggests that the contamination of a churn from the air may be of considerable importance.

The numbers of organisms falling from the air were studied by exposing beef infusion agar plates and acidified (pH 3.5 with lactic acid) malt agar plates about 5 feet from the floor near one of the churns, on the roller of churn A (see Part 2) midway between the middle and one of the ends, and on the shelf of churn B (see Part 2) near the door midway between the middle and one of the ends; when the plates were exposed in a churn the door opening was on the side. Usually the plates were exposed for 30 minutes outside a churn and for 2 hours inside. After incubating the plates for 4 days at room temperature (commonly about 70° F.), the colonies were counted and the results expressed as the number of colonies developing on a 90 mm. plate per hour of exposure. Since detailed examinations of the colonies on the infusion agar plates were not made, the bacterial counts presumably included the yeasts also; however, the numbers of yeasts, compared with the numbers of bacteria, were small. Commonly, plates were exposed in a churn just after it had been treated with hot water and allowed to dry, but, occasionally, a day or two elapsed between the treatment and the exposure. In a few instances the churn was protected during the exposure by a muslin covered frame which fitted into the door opening. The results obtained are presented in table IX.

Fifty-seven exposures were made near one of the churns. The bacteria falling per plate per hour ranged from 4 to 754 and averaged 169; the yeasts varied from 0 to 70 and averaged 7.3, while the molds ranged from 2 to 328 and averaged 29. With 42 exposures made in churn A, the bacteria falling per plate per hour varied from 1 to 234 and averaged 29; the yeasts ranged from 0 to 6 and averaged 1.6, and the molds varied from 0 to 62 and averaged 13.3. With 48 exposures made in churn B, the bacteria falling per plate per hour varied from 1 to 226

TABLE IX. BACTERIA, YEASTS AND MOLDS FALLING PER HOUR ON 90 mm. PETRI PLATES

Date	Hour	Creamery air			Inside churn A			Inside churn B		
		Bact.	Yeasts	Molds	Bact.	Yeasts	Molds	Bact.	Yeasts	Molds
9/28/31	10 A. M.	255	6	18	.....	.....	.....	49	2	14
9/30/31	10 A. M.	58	2	52	5	2	11	14	1	11
10/ 1/31	10 A. M.	14	1	35	.....	.....	.....	2	1	19
10/ 5/31	3 P. M.	142	54	328	.....	.....	.....	43	6	52
10/ 8/31	7 P. M.	18	6	28	35	1	4	11	1	12
10/ 9/31	7 P. M.	16	2	40	9	1	15	9	2	22
10/10/31	7 P. M.	54	2	50	27	2	62	13	1	12
10/12/31	7 P. M.	14	2	22	62	6	30	46	5	27
10/13/31	7 P. M.	26	14	30	36	6	15	13	2	6
10/14/31	7 P. M.	28	2	24	76	4	32	19	0	16
10/15/31	7 P. M.	14	2	36	21	0	9	10	2	18
10/16/31	7 P. M.	16	0	76	21	2	40	24	2	28
10/19/31	7 P. M.	30	0	32	51	2	47	10	2	10
10/20/31	7 P. M.	30	2	38	26	1	6	30	0	8
10/21/31	7 P. M.	.....	.....	.....	6	1	9	5	0	15
10/26/31	7 P. M.	26	2	58	27	4	31	17	4	39
10/27/31	7 P. M.	48	12	76	25	5	31	10	4	29
10/30/31	7 P. M.	22	5	13	5	1	6	10	1	5
11/ 2/31	7 P. M.	18	2	54	39	4	13	17	2	3
11/ 3/31	7 P. M.	34	8	98	.....	.....	.....	8	0	32
11/ 4/31	7 P. M.	24	70	104	1	2	34	5	1	27
11/ 5/31	7 P. M.	54	8	56	1	5	24	.....	.....	.....
11/11/31	7 P. M.	24	6	18	1	3	4	6	3	13
11/12/31	7 P. M.	4	8	28	4	1	9	1	1	8
11/13/31	7 P. M.	8	8	34	4	3	29	5	3	16
11/16/31	7 P. M.	4	4	36	14	1	18	1	3	3
11/18/31	7 P. M.	12	2	8	3	1	6	4	0	4
11/20/31	7 P. M.	.....	.....	.....	1	0	8	1	0	3
12/ 2/31	7 P. M.	19	2	10	5	0	5	10	1	8
12/ 4/31	2 P. M.	30	0	10	2	0	2	4	0	9
12/ 7/31	2 P. M.	24	0	18	15	1	8	21	3	9
12/ 8/31	2 P. M.	11	5	13	4	1	7	3	0	9
12/10/31	2 P. M.	24	1	15	26	1	2	24	0	1
12/14/31	1 P. M.	.....	.....	.....	34	2	8	.....	.....	.....
12/15/31	2 P. M.	22	0	6	2*	1	6	2*	0	1
12/17/31	3 P. M.	460	3	12	14*	1	2	8*	0	1
1/ 7/32	3 P. M.	579	0	2	3*	0	0	4*	0	0
1/ 9/32	1 P. M.	410	1	2	.....	.....	.....	.....	.....	.....
1/11/32	3 P. M.	265	2	4	95	1	5	181	1	5
1/13/32	2 P. M.	52	8	6	.....	.....	.....	3*	0	2
1/15/32	2 P. M.	274	1	5	.....	.....	.....	1	1	0
1/21/32	1 P. M.	103	1	2	10	0	6	93	1	5
1/27/32	1 P. M.	.....	.....	.....	64	0	0	.....	.....	.....
1/28/32	2 P. M.	465	34	12	.....	.....	.....	.....	.....	.....
2/ 1/32	2 P. M.	112	1	9	.....	.....	.....	.....	.....	.....
2/ 3/32	3 P. M.	110	3	5	.....	.....	.....	2	0	3
2/ 6/32	2 P. M.	264	1	6	33*	0	2	.....	.....	.....
2/15/32	2 P. M.	443	3	2	.....	.....	.....	.....	.....	.....
2/18/32	2 P. M.	188	2	4	22	0	3	22	0	1
2/24/32	3 P. M.	271	28	3	5*	0	1	.....	.....	.....
2/26/32	2 P. M.	195	9	5	.....	.....	.....	.....	.....	.....
2/27/32	2 P. M.	541	4	15	.....	.....	.....	.....	.....	.....
3/ 3/32	3 P. M.	259	2	3	86	0	2	153	1	4
3/ 7/32	2 P. M.	659	10	17	.....	.....	.....	144	2	5
3/11/32	2 P. M.	428	20	15	.....	.....	.....	83	1	6
3/14/32	2 P. M.	200	1	5	.....	.....	.....	.....	.....	.....
3/18/32	2 P. M.	608	3	8	63	1	3	.....	.....	.....
3/24/32	2 P. M.	343	26	14	.....	.....	.....	24	1	4
3/28/32	3 P. M.	478	13	15	234	1	2	217	1	6
4/ 1/32	3 P. M.	754	4	10	.....	.....	.....	226	2	7
4/ 4/32	2 P. M.	67	0	10	.....	.....	.....	14	0	1
Average		169	7.3	29.0	29	1.6	13.3	34	1.3	11.2

* Door covered with muslin.

and averaged 34; the yeasts ranged from 0 to 5 and averaged 1.3, and the molds varied from 0 to 52 and averaged 11.2.

In seven comparisons one set of plates was exposed in a churn protected with a muslin door covering while another set was exposed in an unprotected churn. Churn A was the protected churn in two trials and churn B in five. The results secured are presented in table X.

TABLE X. COMPARISON OF BACTERIA, YEASTS AND MOLDS FALLING PER HOUR ON A 90 mm. PETRI DISH IN A CHURN PROTECTED WITH A MUSLIN DOOR COVERING AND IN AN UNPROTECTED CHURN

	Plates exposed in a churn protected with muslin door covering				Plates exposed in an unprotected churn			
Date	Churn	Bacteria	Yeasts	Molds	Churn	Bacteria	Yeasts	Molds
1/28/32	A	14	0	1	B	88	29	6
2/ 1/32	B	3	0	1	A	73	0	6
2/15/32	B	6	0	1	A	83	1	2
2/26/32	B	13	0	2	A	45	2	5
2/27/32	B	6	0	2	A	427	1	8
3/14/32	B	6	0	0	A	112	0	0
4/ 8/32	A	29	1	1	B	292	0	3
Average		11	.1	1.1		160	4.7	4.3

With the protected churn the bacteria falling per plate per hour ranged from 3 to 29 and averaged 11, while with the unprotected churn they varied from 45 to 427 and averaged 160. There were no yeasts falling in the protected churn in six trials and 1 in the other, while in the unprotected churn the numbers ranged from 0 to 29 and averaged 4.7. The molds falling in the protected churn ranged from 0 to 2 and averaged 1.1, while in the unprotected churn they varied from 0 to 8 and averaged 4.3.

The bacteria falling on the plates exposed both outside and inside a churn represented a variety of types and commonly included a large proportion of chromogenic micrococci and organisms belonging to the genus Bacillus.

The general results show that considerable numbers of organisms were falling from the air in a butter plant and that the numbers falling inside a churn having the door on the side were smaller than the numbers falling outside. Commonly, the numbers of bacteria falling were larger than the numbers of yeasts or molds, and the numbers of molds were larger than the numbers of yeasts.

PART 6. GENERAL OBSERVATIONS ON THE CONTAMINATION FROM CHURNS

In the studies at the Iowa Agricultural Experiment Station on the bacteriology of butter, instances have been encountered in which serious contamination of butter occurred from churns that were being treated carefully, the treatment involving the

use of washing powder, an abundance of very hot water and occasional rinsing with a suspension of lime. Usually, contamination from the churns was first suspected when the bacterial counts secured on experimental butter were higher than was expected, considering the bacterial content of the cream. Commonly, the bacteria in the butter were of a variety of types and very definitely included types that could not be detected in the cream. The general condition of the churns was such that extensive contamination from them was not expected. In some of the instances, examinations, through a series of trials, of the butter and of the cream from which is was obtained indicated that the churn was the source of the contamination, even when it was given unusually thorough treatment between trials.

One instance of the serious contamination of butter from the churn was investigated in considerable detail. After several examinations of the churn had revealed nothing unusual, one end of a shelf support was found so loose that it could be moved through a distance of about three-eighths of an inch. At the time it seemed unlikely that the support would move during the churning process but, undoubtedly, when a strain was thrown on the churn, for example, during the working of the butter, movement of the support could occur. Beneath the end of the support there was a mass of material that had a very objectionable odor and contained large numbers of bacteria. The replacement of the shelf support with a new one that was held firmly in position resulted in a great reduction in the contamination from the churn.

There are, undoubtedly, a number of points in a churn at which contamination foci can be established, the extensive growth of organisms occur and then the organisms be forced out when the churn is in use. The suggestions of various investigators include packing, openings between pieces of wood, etc.

Undoubtedly certain contamination foci in churns could be so well protected that the organisms in them would largely survive either hot water or chemical treatment of the churns. It seems probable that when material is forced from these foci, as a result of a strain on the churns, moisture and nutrients would be drawn into the foci when the strain is relieved. In this way the continued growth of organisms would be possible. With the contamination foci well protected and the remainder of the churn relatively free from organisms, both the rinse and agar disc methods of examining churns would show a satisfactory microbiological condition.

The general observations on contamination from churns indicate that contamination foci can be established in churns that are given careful treatment.

PART 7. INFLUENCE OF THE CONTAMINATION FROM CHURNS ON THE KEEPING QUALITIES OF BUTTER

While the contamination of butter with a considerable number of organisms from the churn is definitely objectionable from the standpoint of proper plant operation, not all of the organisms added are of importance in causing deterioration in the butter and, accordingly, studies on the influence of the contamination from the churn on the keeping qualities of butter were carried out.

(a) INFLUENCE OF ORGANISMS ISOLATED FROM CHURNS ON THE KEEPING QUALITIES OF BUTTER

In order to determine the influence of the general types of bacteria present in churns on the keeping qualities of butter, churnings were made on a laboratory scale, the cream being inoculated just before churning with organisms isolated from churns. The inoculations were regularly very heavy since many of the organisms in cream are carried away in the buttermilk when the cream is churned, and the object was to determine whether or not the organisms could produce changes in butter under favorable conditions.

Pure cultures of the organisms were spread on beef infusion agar plates, the plates incubated for 2 days at 86° F. and the growth on each plate suspended in about 10 ml. of sterile water; when mixed cultures were to be used the organisms were grown in combination on the agar plates. The cream was prepared by placing about 250 ml. portions in quart glass jars and pasteurizing it at 180° F. for 20 minutes in a water bath. After cooling to about 50° F. by running cold water into the bath, each portion was inoculated with a suspension of organisms from a plate, one portion in each series being kept as a control, and churned; the butter was washed with sterile water and worked with sterile equipment. The unsalted butter was packed in sterile glass jars, each holding about one-third of a pound, and protected by a piece of parchment paper under a metal screw cap. It was stored at about 59° F., in order to favor the development of the organisms, and examined for definite defects at intervals of about a week for a period of about 8 weeks; no significance was attached to the results unless the control butter remained free from serious defects.

Sixty-one pure cultures of bacteria and five mixed cultures were used in studying the effects of organisms isolated from churns on the keeping qualities of butter. Each mixed culture was prepared by streaking, on an agar plate, from 15 to 20 colonies representing the various types of bacteria on an agar disc. All of the organisms were aerobic and grew well at 70° F. It should be noted that the cream from which the butter was

made was very heavily inoculated and that the butter was unsalted and was held at about 59° F. so that conditions were very favorable for the growth of bacteria in the butter.

Thirty-two of the organisms belonged to the genus Bacillus. In general, these organisms produced changes in butter slowly, and they were not extensive, although with 13 of the cultures the defect produced was eventually conspicuous. Three of the cultures produced little change, while nine produced a nutty flavor, nine an unclean flavor, five rancidity, four cheesiness or surface taint and two a malty flavor.

Seventeen of the organisms were non-spore forming rods that undoubtedly belonged to several genera. The changes produced in butter by these organisms were usually rapid and extensive. Two of the cultures produced little change, twelve produced an unclean flavor, one rancidity, one a bitter flavor and one a fruity flavor.

Twelve of the organisms were micrococci and, in general, these produced rapid and extensive changes. Two cultures produced little change, six produced rancidity, two an unclean flavor, one a nutty flavor and one cheesiness.

Each of the five mixed cultures produced changes in butter very rapidly and the defects were generally very pronounced. One produced rancidity, one surface taint, one a malty flavor, one a musty flavor and one an old cream flavor.

The results secured with the organisms isolated from churns indicate that most of them were capable of bringing about changes in butter under conditions favorable for their activity. The changes produced were of various types.

(b) INFLUENCE OF A HIGHLY CONTAMINATED CHURN ON THE KEEPING QUALITIES OF BUTTER

The general procedure for determining the influence of a highly contaminated churn on the keeping qualities of the butter made in it was as follows: After the highly contaminated churn had been examined for the number of organisms present, one-half of a lot of pasteurized cream was churned in it; the churn was then thoroughly washed and treated with hot water; the number of organisms remaining was determined and the other half of the cream then churned. Both unsalted and salted butter from each churning were held at 45° F. and at 32° F. in order to compare the keeping qualities of the butter churned before and after the cleaning of the churn.

The churns used were small experimental churns of 70 pounds capacity and 75 pounds of cream were used for each churning. Churn C was used in only two trials because the butter could not be worked satisfactorily in it, and the studies were completed with churn D. A high contamination was established in

a churn by careless treatment; this consisted of rinsing out the fat with water at about 120° F. (following the removal of a churning of butter), filling the churn one-third to one-half full of water at about 170° F., revolving for 5 minutes and then draining and allowing to stand; the drying was slow and incomplete because the treatment of the churn was hurried. In order to contaminate the churn heavily when it had been washed carefully, a small amount of buttermilk was agitated in it for a short period and the churn then carelessly treated. Following the use of a churn in a contaminated condition it was washed thoroughly and then filled nearly full of water at 200° F. or over and revolved for 30 minutes. With the rinse method on the clean churn, water at about 33° F. was used in order to cool the churn following the hot water treatment.

In most of the trials the cream used was taken from a vat of selected sweet cream pasteurized at 145° F. for 30 minutes. When no butter culture was used, the cream was drawn from the vat immediately after pasteurizing and cooling and stored overnight at about 40° F. When butter culture was used, 7 percent was mixed with the cream in the vat, the mixture held cold overnight and the cream for the experimental churnings drawn in the morning. In a number of trials a pasteurization exposure of 155° F. for 30 minutes was desired and the 150 pounds of cream needed were pasteurized in a small vat; after cooling the cream to about 36° F. it was drawn into two 10-gallon cans and stored at about 40° F. overnight. The cream to be churned in the contaminated churn was warmed to from 48° to 52° F. just before churning, while that to be churned in the clean churn was warmed to only from 38° to 42° F. because of the additional warming that occurred in the churn as a result of treatment with hot water.

The butter in a churning was washed thoroughly with tap water (at about 54° F.), worked into a homogeneous mass and the unsalted sample taken. After incorporating about 2.5 percent of salt in the remainder of the butter the sample of salted butter was taken. The butter was held in sterile glass jars holding about one-third pound, the butter being protected by a parchment paper under the metal screw caps. The samples held at 32° F. were scored at irregular intervals, and those held at 45° F. were scored each week.

The cream ready for the churn and the fresh unsalted butter were plated on beef infusion agar and on malt agar acidulated to pH 3.5 with lactic acid; after storage for from 21 to 63 days at 45° F. the unsalted butter was again plated on beef infusion agar. All the plates were incubated at room temperature (about 70° F.) for 4 days and counted with the aid of a hand lens.

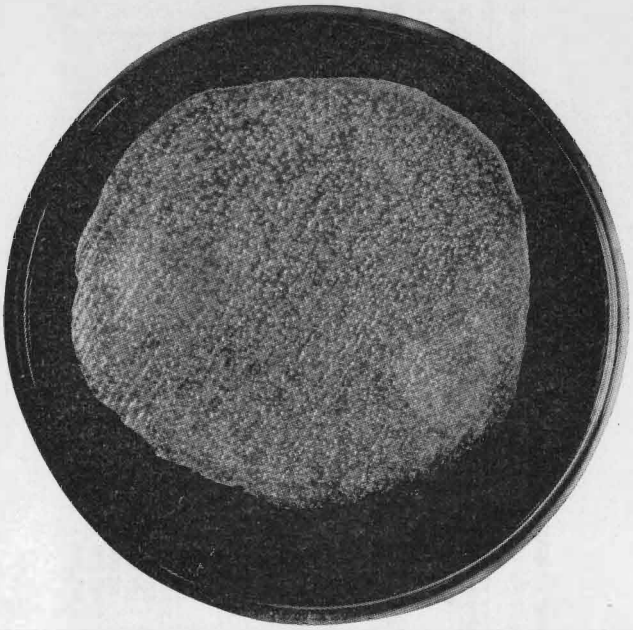

Fig. 7. Agar disc from the shelf of a highly contaminated experimental churn just before the churn was used.

Microscopic counts, according to the method devised by Hammer and Nelson (6), were made on the fresh unsalted butter and on the samples of salted and unsalted butter after storage for 7 days at room temperature (about 70° F.).

The counts secured on the highly contaminated and clean churns just prior to use are given in table XI.

According to the agar disc method the contaminated churns contained from 12 to more than 2,000 bacteria per sq. cm. with an average of 710, from 0 to 0.80 yeasts with an average of 0.14 and from 0.01 to 0.70 molds with an average of 0.16. Agar disc counts were not made on the clean churns because the temperature and moisture in a churn just after treatment with hot water interfere with the preparation of satisfactory discs (7). Figure 7 shows an agar disc from the shelf of a highly contaminated churn just before the churn was used.

With the rinse method the bacterial counts on the contaminated churns ranged from 21,000 to 6,500,000 per ml. and averaged 1,587,000, while with the clean churns they ranged from 1,100 to 17,300 per ml. and averaged 4,531. The yeast counts on the contaminated churns ranged from 0 to 70 per ml. and

TABLE XI. COUNTS ON CONTAMINATED AND CLEAN CHURNS JUST PRIOR TO USE

Trial	Date	Agar disc counts on contaminated churn			Plate counts on rinse water							
					Contaminated churn				Clean churn			
		Organisms per sq. cm.			Water used for rinse	Water after exposure to churn Organisms per ml.			Water used for rinse	Water after exposure to churn Organisms per ml.		
		Bacteria	Yeasts	Molds	Bacteria per ml.	Bacteria	Yeasts	Molds	Bacteria per ml.	Bacteria	Yeasts	Molds
1	12/18/31	>2,000	.80	.70	160	2,300,000	45	25	162	1,750	0	7
2	12/21/31	>2,000	.08	.32	433	6,000,000	3	20	162	2,900	0	6
3	12/22/31	593	.05	.10	80	141,000	26	18	72	4,950	4	4
4	1/19/32	681	.23	.27	33	70,000	4	1	22	1,270	1	0
5	2/ 2/32	12	.01	.05	36	42,000	4	1	20	1,630	2	0
6	2/16/32	40	.04	.09	22	41,000	0	1	15	1,100	2	0
7	2/23/32	850	.27	.17	10	1,830,000	10	1	36	2,100	3	0
8	3/ 1/32	>2,000	.39	.20	16	1,790,000	50	0	44	4,200	23	0
9	3/ 8/32	460	.01	.06	128	21,000	0	2	19	2,700	0	3
10	3/15/32	75	0	.03	28	295,000	70	4	47	3,150	3	1
11	3/22/32	>1,000	.10	.36	22	240,000	23	0	28	6,100	9	0
12	3/31/32	800	.16	.14	40	5,500,000	49	1	42	12,800	2	0
13	4/ 5/32	416	.01	.01	21	370,000	19	0	20	4,000	2	1
14	4/12/32	26	0	.02	59	177,000	13	14	48	17,300	12	1
15	4/19/32	31	0	.01	10	82,000	11	9	63	5,450	6	2
16	4/26/32	369	.01	.08	54	6,500,000	8	1	43	1,100	2	3
Average		710	.14	.16	72	1,587,000	20.9	6.1	53	4,531	4.4	1.8

averaged 20.9 while with the clean churns they ranged from 0 to 23 per ml. and averaged 4.4. The mold counts on the contaminated churns ranged from 0 to 25 per ml. and averaged 6.1, while with the clean churns they ranged from 0 to 7 per ml. and averaged 1.8. The bacteria in the rinse water from the contaminated churns were of a variety of types, while those in the rinse water from the clean churns were of few types and belonged very largely to the genus Bacillus.

The plate counts on the cream ready for the churn and on the fresh unsalted butter from the contaminated and from the clean churns are presented in table XII. In the first 10 trials the pasteurization exposures were 145° F. for 30 minutes, while in the last 6 they were 155° F. for 30 minutes. In trials 3 and 10, butter culture (7 percent) was used in the cream.

TABLE XII. PLATE COUNTS ON THE CREAM READY FOR THE CHURN AND ON THE FRESH UNSALTED BUTTER

Trial	Plate count on								
	Cream			Fresh unsalted butter from					
				Contaminated churn			Clean churn		
	Organisms per ml.			Organisms per ml.			Organisms per ml.		
	Bacteria	Yeasts	Molds	Bacteria	Yeasts	Molds	Bacteria	Yeasts	Molds
1	271,000	0	4	1,730,000	14	2	53,000	1	2
2	47,000	0	0	610,000	0	0	12,000	0	0
3*	15,000,000	0	1	800,000	10	0	890,000	0	0
4	1,900,000	5	1	74,000	3	2	120,000	2	0
5	125,000	59	4	36,000	9	3	12,600	3	1
6	67,500	2	0	10,400	2	0	2,900	2	0
7	80,000	2	1	370,000	3	1	11,800	2	1
8	69,000	0	10	145,000	3	0	14,400	1	1
9	132,000	1	0	57,000	2	0	14,250	2	0
10*	30,200,000	0	0	360,000	11	4	650,000	7	1
11	40,000	1	0	341,000	29	0	8,000	1	0
12	204,000	0	0	400,000	24	0	27,500	4	0
13	62,000	0	0	35,000	13	0	5,700	0	0
14	3,100	0	0	8,000	4	2	2,800	1	1
15	4,700	0	0	22,000	8	4	3,550	0	0
16	48,000	0	1	49,000	1	4	15,800	0	2
Average	3,016,000	4.4	1.4	315,500	8.5	1.4	115,300	1.6	.6
**Average of 14 trials	218,000	5.0	1.5	277,700	8.2	1.3	21,740	1.4	.6

* Cream with culture added.
** Cream without culture added.

Excluding the two trials in which culture was used, the average bacterial count on the cream pasteurized at 145° F. was 336,500 per ml., while the average count on the cream pasteurized at 155° F. was 60,300 per ml. The cream pasteurized at 145° F. sometimes contained significant numbers of yeasts and molds while these organisms were practically eliminated in the cream pasteurized at 155° F. It should be noted that the cream pasteurized at the lower exposure was handled in larger volumes than that pasteurized at the higher exposure.

In the 14 trials in which the cream was churned without culture, the bacterial count on the butter from the contaminated churn was higher than that on the butter from the clean churn in every case except one, and often the difference was large. In this instance (trial 4) the count on the butter from the clean churn was higher than in any of the other trials in which culture was not used, while the count on the butter from the contaminated churn was comparatively low; the data in table XI show that in this trial the contaminated churn contained a smaller number of organisms than those in some of the other trials. The number of organisms in the butter from the clean churn was also larger than the number in the butter from the contaminated churn in the two trials in which culture was used, but in one of these the difference was not significant. In all the trials the bacterial count on the butter from the clean churn was lower than the count on the cream from which it was churned, while the count on the butter from the contaminated churn was higher than the count on the cream in 9 of the 16 trials; this relationship shows that a contaminated churn may have a very striking effect on the bacterial content of butter churned in it. Figures 8 and 9 illustrate the influence of a contaminated churn on the bacterial content of butter.

The unsalted butter from the contaminated churn often had higher yeast and mold counts than that from the clean churn; the numbers of yeasts and molds in the butter from the contaminated churn were relatively high in several cases while the numbers in the butter from the clean churn were commonly low. The yeast and mold counts on the butter from the contaminated churn were generally higher than the counts on the cream, while the yeast and mold counts on the butter from the clean churn were frequently lower than the counts on the cream.

In the trials where the high pasteurization temperature was used (trials 11 to 16, inclusive), the yeast and mold counts on the cream and on the butter from the clean churn were insignificant, whereas the yeasts and molds in the butter from the contaminated churn were numerous enough to suggest their derivation from the churn.

The plate counts on the unsalted butter after storage at 45° F. for from 21 to 63 days are not presented in the tables. In every case there was a large increase in the numbers of bacteria in the butter during the holding period. After storage the counts on the butter from the contaminated churns ranged from 10,800,000 to 126,000,000 per ml. and averaged 50,288,000, while those on the butter from the clean churns ranged from 210,000 to 378,000,000 per ml. and averaged 61,763,000. One excessively high count is largely responsible for the high average on the

butter from the clean churns; the counts on the butter from the contaminated churns had a logarithmic average of 39,460,000 per ml., while those on the butter from the clean churns had a logarithmic average of 21,650,000. After storage the butter from the contaminated churn had a higher bacterial count than that from the clean churn in 10 of the 16 trials, the butter from the clean churn had the higher count in 5 trials and there was practically no difference in 1 trial.

The microscopic counts are also omitted from the tables. The counts on the fresh unsalted butter from the contaminated churns ranged from 471,000 to 43,000,000 per ml. and averaged 10,779,000, while those on the butter from the clean churns ranged from 197,000 to 46,000,000 per ml. and averaged 9,455,000. The butter from the contaminated churn had a higher bacterial count than that from the clean churn in all but 4 of the 16 trials and in 3 of these, including 1 of the trials in which culture was used, the differences were insignificant. After storage for 7 days at about 70° F. the counts on the salted butter from the contaminated churns ranged from 249,000 to 48,000,000 per ml. and averaged 13,208,000, while those on the salted butter from the clean churns ranged from 141,000 to 56,000,000 per ml. and averaged 7,059,000; the butter from the contaminated churn had the higher count in all but 4 of the 16 trials, and in 3 of these the differences were very small. After storage the counts on the unsalted butter from the contaminated churns ranged from 12,000,000 to 278,000,000 per ml. and averaged 98,188,000, while those on the unsalted butter from the clean churns ranged from 8,500,000 to 292,000,000 per ml. and averaged 67,531,000; the butter from the contaminated churn had the higher count in 12 of the 16 trials, but in 3 of the remaining trials the counts on the butter from the clean churn were much larger than those on the butter from the contaminated churn. In every trial the bacterial count of the unsalted butter was larger after storage than that of the salted butter from the same churning.

The average scores on the salted and unsalted butter from the contaminated and from the clean churns after various holding periods at 45° or 32° F. are given in table XIII; the butter held at 32° F. was scored at irregular intervals so that the average scores do not always represent the entire 16 samples, and some of the fluctuations in the average scores may be due to this. The data presented in table XIII are shown graphically in figs. 10 and 11.

The scores on the salted butter stored at 45° F. indicate that there was no significant difference between the keeping qualities of the butter from the contaminated churns and those of

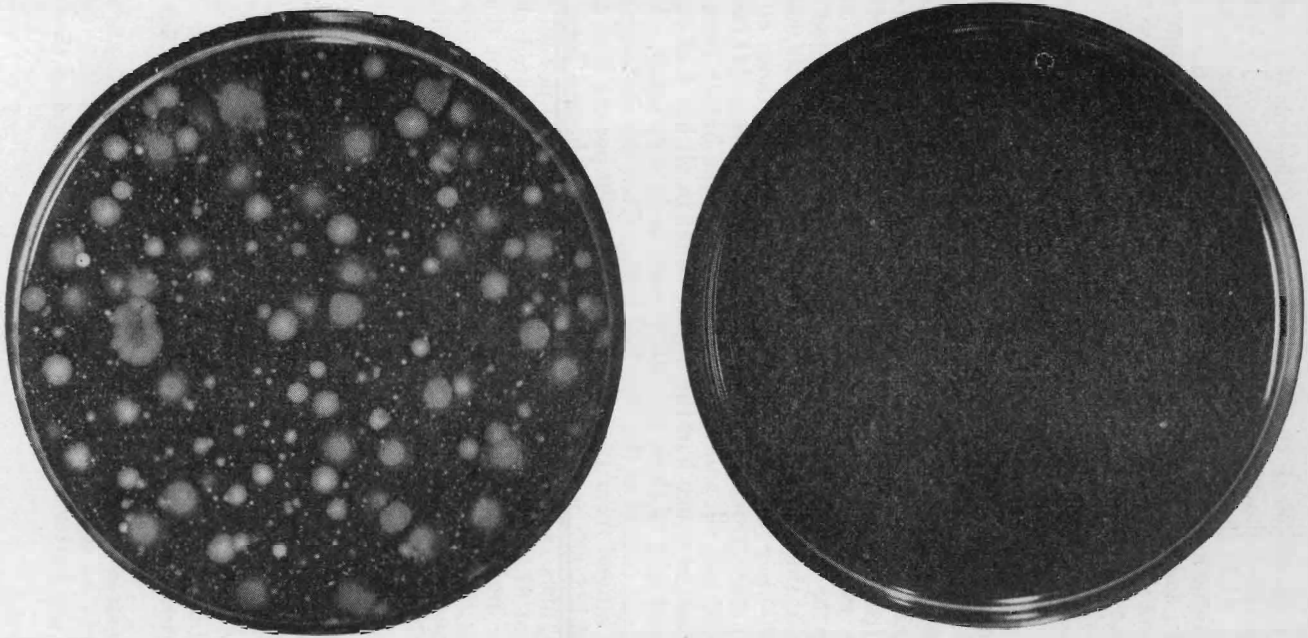

Fig. 8. Bacteria in unsalted butter from contaminated and from clean churns, the cream used being from the same source; 1-1000 ml. of butter was used per plate.

Fig. 9. Bacteria in unsalted butter from contaminated and from clean churns, the cream used being from the same source; 1-100 ml. of butter was used per plate.

TABLE XIII. AVERAGE SCORES OF THE BUTTER FROM CONTAMINATED CHURNS AND FROM CLEAN CHURNS

16 trials

Days stored	Butter stored at 45° F.				Butter stored at 32° F.			
	Salted butter from		Unsalted butter from		Salted butter from		Unsalted butter from	
	Contaminated churns	Clean churns	Contaminated churns	Clean churns	Contaminated churns	Clean churns	Contaminated churns	Clean churns
2	92.4	92.5	92.4	92.4	92.4	92.5	92.4	92.4
9	92.4	92.5	91.0	92.0	.....	.....	.....	.....
16	91.9	92.1	89.3	90.5	92.2	92.7	91.9	92.0
23	91.4	91.6	88.3	89.7	.....	.....	.....	.....
30	91.5	91.6	87.3	88.9	91.8	91.8	89.9	91.8
37	90.9	91.0	86.9	88.8	.....	.....	.....	.....
44	90.8	90.9	86.6	88.7	91.8	91.8	89.8	91.8
51	90.4	90.8	86.6	88.9	.....	.....	.....	.....
58	90.3	90.4	86.5	88.7	91.0	91.0	88.8	90.5
65	90.3	90.5	86.2	88.3	.....	.....	.....	.....
72	90.5	90.5	86.0	88.0	90.7	91.3	88.8	89.6
86	.....	.....	.....	.....	90.0	90.0	89.0	89.5
100	.....	.....	.....	.....	89.6	89.8	87.1	88.6

the butter from the clean churns. The changes that took place in the butter were gradual and none of the samples showed pronounced defects even after extended holding. The scores on

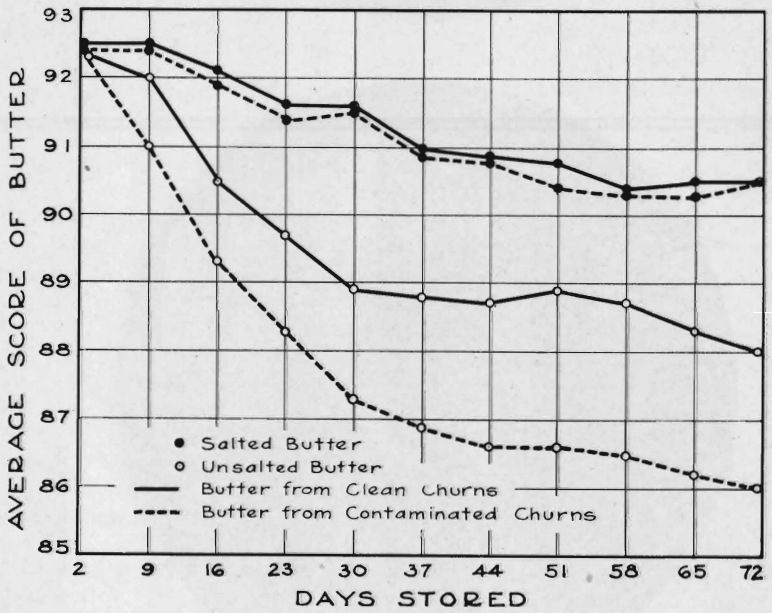

Fig. 10. Deterioration at 45° F. of butter from contaminated churns and from clean churns; average of 16 trials.

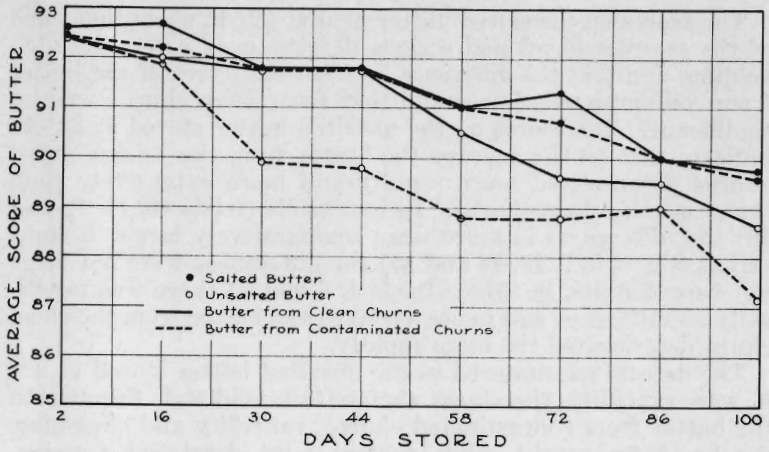

Fig. 11. Deterioration at 32° F. of butter from contaminated churns and from clean churns; average of 16 trials.

the unsalted butter stored at 45° F. show that on the average the butter from the clean churns possessed keeping qualities distinctly superior to those of the butter from the contaminated churns. In all except 2 of the 16 trials (trials 4 and 8) the butter from the contaminated churn deteriorated more rapidly than that from the clean churn; in one of the two trials (trial 4) the pasteurized cream contained an unusually large number of organisms which may have been a factor in the deterioration of both lots of butter. In 9 trials (trials 5, 7, 9, 10, 11, 12, 13, 14 and 16) the butter from the contaminated churns showed distinctly poorer keeping qualities than that from the clean churns, while in 5 trials (trials 1, 2, 3, 6 and 15) the differences in scores were not so large. The development of defects was generally much more rapid and more extensive in the butter from the contaminated churn than in that from the clean churn, the difference in score sometimes being 4 or 5 points. There were large differences in scores in 5 trials (trials 9, 11, 12, 13 and 14) and four of these were among the 6 trials in which a high pasteurization exposure was used with the cream.

The most common defects that developed in the unsalted butter stored at 45° F. were rancidity, cheesiness and fruity and unclean flavors. In the butter from contaminated churns, rancidity was a very common defect while, when extensive deterioration took place in the butter from clean churns, cheesiness was the common defect. Fruity flavors were encountered in both the butter from the contaminated churns and that from the clean churns.

The scores on the salted butter held at 32° F. show that none of the samples developed serious defects, even after extended holding, and that the difference between the scores of the butter from contaminated churns and that from clean churns was insignificant. The scores on the unsalted butter stored at 32° F. indicate that on the average the butter from the contaminated churns deteriorated more rapidly and more extensively than that from the clean churns. In four trials (trials 10, 11, 12 and 16) the differences in score were comparatively large, in eight (trials 1, 2, 3, 5, 7, 13, 14 and 15) the differences were not large but very definite, in three (trials 4, 6 and 9) there was practically no difference and in one (trial 8) the butter from the clean churn deteriorated the more rapidly.

The defects encountered in the unsalted butter stored at 32° F. were rancidity, cheesiness, surface taint and stale flavors. In the butter from contaminated churns, rancidity and cheesiness developed frequently, while surface taint developed occasionally; when extensive deterioration occurred in the butter from clean churns cheesiness was the most common defect. When the holding period was from 1 to 3 months at 32° F., rancidity was rather uncommon, but after holding for from 7 to 10 months at this temperature, 12 of the 16 samples of unsalted butter from the contaminated churn were rancid, while only 1 sample from the clean churn showed rancidity.

The data presented in table XIII emphasize the great value of salt in preventing the deterioration of butter at either 45° or 32° F. They also show the importance of temperature in this connection since salted and unsalted butter from both contaminated and clean churns kept better, on the average, at 32° than at 45° F.

The general results secured indicate that the highly contaminated churns had a very definite effect on the keeping qualities of butter churned in it when the butter was unsalted and was held at 32° or 45° F.; when the butter contained 2.5 percent salt the effect was insignificant.

DISCUSSION OF RESULTS

PART 1

A variation in the microbiological condition of the churns in Iowa butter plants would be expected from the variation in the general condition of these plants. When a plant is well cared for, the churn usually receives the attention due it while, when a plant is poorly cared for, the churn also is commonly neglected. In the churns examined there were many more bacteria than yeasts or molds; this relationship is in agreement with the observations and suggestions of various investigators (3, 9, 13, 14, 18 and 19). Although the development of yeasts or molds in butter may be responsible for serious defects, in general, bacteria are a greater factor in the deterioration of butter than yeasts or molds.

The predominance of resistant types, such as organisms belonging to the genus Bacillus and certain micrococci, in churns containing comparatively few bacteria was presumably due to the efficiency of the attempts to destroy organisms in the churns.

The objectionable odors commonly present in churns that were moist show the necessity of having churns dry if the development of organisms in them is to be prevented. Presumably, the greatest factor in controlling the growth of organisms in a churn is proper drying.

PART 2

The efficiency of regular treatment of churns with hot water in keeping the numbers of organisms comparatively low and largely limiting those present to heat resistant types is in agreement with the value of heat in the treatment of metal dairy equipment. Various investigators (5, 18 and 21) have concluded that the hot water treatment of churns is a satisfactory procedure, but it is evident from the studies reported (9, 18, 19 and 21) that certain bacteria survive such treatment. Because of the difficulties inherent in the treatment of churns as a result of the use of wood in the construction, it is probable that churns will continue to be a more important source of the organisms in butter than the equipment made of metal.

The failure of the counts on the churns regularly treated with hot water to show a seasonal distribution suggests that in such churns there is no extensive growth of organisms and that the organisms found are largely those left by the washing and those falling in from the air. With long intervals between treatments, the probabilities of growth are undoubtedly much greater than with short intervals.

PART 3

The definite reductions, in the numbers of bacteria in churns treated with hot water, that were secured through the use of a solution of sodium hypochlorite, chlorinated lime or calcium hypochlorite would be expected from the general value of these reagents for the destruction of organisms. Various investigators (3, 9, 19 and 23) have found that chemical treatment of churns results in pronounced reductions in the numbers of organisms present. Undoubtedly some of the organisms in churns are more or less protected but, presumably, many others are so exposed that they can be reached by solutions used in the churns and their destruction effected if the solutions are sufficiently germicidal.

The failure of a cold, saturated solution of sodium chloride to reduce the numbers of organisms in a churn treated with hot water is in agreement with the general action of such a solution on resistant organisms. Somewhat different results might have been secured if the churn used had been highly contaminated with a variety of organisms. There is also the possibility of a cold sodium chloride solution containing salt-tolerant organisms.

PART 4

The large reductions in the numbers of bacteria in highly contaminated churns as a result of treatment with hot water, a solution of sodium hypochlorite or a solution of a chloramine preparation confirms the general results reported in Parts 2 and 3 on the action of hot water and chlorine compounds on the organisms in churns. Undoubtedly, in a highly contaminated churn some of the organisms are protected better than in a clean churn, but many of them can be destroyed by heat or chemicals. Repeated washing and treatment of highly contaminated churns, when properly carried out, would be expected to gradually reduce the numbers of organisms in a churn to a point beyond which it is difficult to go with any type of treatment. If contamination foci have been established these may be difficult to eliminate and may contribute many organisms to the butter.

The fact that considerable numbers of bacteria and significant numbers of yeasts and molds were present in the rinse water used after the treatment of a churn, while the hypochlorite solution used for the treatment contained few bacteria and no yeasts and molds suggests that there are points in a highly contaminated churn which are not readily reached by a solution. Perhaps some of these points could develop into contamination foci which would be difficult to eliminate, even with careful treatment of the churn. An important step in the eradication of such points is the thorough cleaning of the churn.

Under the conditions employed with the solutions of sodium hypochlorite and the chloramine preparation, the temperature probably had some effect in destroying organisms, especially with the chloramine preparation.

The large number of organisms in the rinse water used before the treatment of the churns illustrates the mechanical removal of organisms in the cleaning of churns and suggests that there may be an advantage in rinsing a churn just before cream is added to it.

PART 5

The development of organisms on plates exposed in a churn suggests that contamination from the air may be of importance in connection with the microbiological condition of a churn. In a dry churn the organisms from the air presumably increase the number present in direct proportion to the number that fall, but in a moist churn they may grow and thus be responsible for large increases in the organisms present. The organisms coming from the air may account for the larger numbers of organisms on the roller and shelf near the door of a churn than on the ends and barrel since the former are apparently in positions where contamination from the air is relatively heavy. It is of interest to note that the comparative numbers of bacteria, yeasts and molds falling from the air are essentially the same as the comparative numbers found in churns.

The smaller numbers of organisms falling on plates exposed in churns having muslin over the door openings than on plates exposed in unprotected churns indicate that the muslin tends to exclude some of the organisms that would otherwise get into the churns. The partial elimination of convection currents in a warm churn may be of importance in this connection.

PART 6

The general observation that contamination foci in churns being given careful treatment may be responsible for the contamination of butter is in agreement with the observations and general conclusions of various investigators (9, 16 and 17). The studies of Macy, Combs and Morrison (16) are especially significant in this connection. Since it seems probable contamination foci may be so well protected that neither the rinse nor agar disc method would indicate the organisms in them, the limitations of these methods of examining churns should be recognized. The final test is the addition of organisms to the butter, but this addition is difficult to determine because of the variable relationship of the number of organisms in the cream and in the butter churned from it. The detection in the butter of species of organisms not found in the cream may be useful in the study of churn contamination.

PART 7

The production of definite defects in unsalted butter at 59° F. by pure cultures of most of the organisms isolated from churns shows the importance of the contamination of butter from this source. The influence of contamination from churns is also evident from the greater deterioration of unsalted butter, at either 32° or 45° F., when it was churned in a contaminated churn than when it was churned in a clean churn; the more extensive development of bacteria in the butter from the contaminated churn than in that from the clean churn suggests the relationship of organisms to the deterioration encountered.

Although there were no significant differences in the keeping qualities of salted butter from contaminated and from clean churns, it should be recognized that the salt concentration was relatively high and, with a lower salt content, differences might have been encountered. There is no sharp line of division, from the standpoint of action on microorganisms, between salted and unsalted butter and there may be less difference between low salted and unsalted butter than between low salted and high salted butter.

The influence of temperature on the rate of deterioration of the butter was probably due to the effect of temperature on the growth of the organisms. Presumably, with temperatures sufficiently low, the butter from the contaminated churn would keep as well as that from the clean churn. In commercial channels, however, butter often encounters comparatively high temperatures when it is in the hands of the retailer and consumer, and it should be manufactured to withstand these conditions.

The greater frequency with which rancidity was encountered in the butter from the contaminated churns than in that from the clean churns suggests that organisms from contaminated churns often include types capable of producing this defect.

GENERAL

The results obtained show that with both hot water treatment and chlorine treatment organisms remained in churns and could be demonstrated by either the agar disc or the rinse method. Since the hot water and the chlorine solutions used in the treatments regularly contained bacteria, they cannot be expected to destroy all of the organisms in a churn. The predominance of bacteria belonging to the genus Bacillus in churns treated with hot water makes it unlikely that all of these organisms can be destroyed by any heat treatment applicable to churns.

In the treatment of churns the improbability of destroying all the organisms present should be recognized. This does not imply that churns should be treated carelessly, since the organisms coming from contaminated churns have a definite influence

on the keeping qualities of unsalted butter and, presumably, of butter low in salt. Since the data presented indicate clearly that unsalted butter churned from carefully pasteurized cream in churns containing comparatively few organisms deteriorates rather rapidly at temperatures satisfactory for the growth of organisms, it is probable that the question of the keeping qualities of butter is not to be solved through the use of carefully treated churns alone.

Both heat and chlorine compounds undoubtedly have an effect on the wood of churns and the same is presumably true of washing powders and lime. This effect, however, should not be used as an argument against the proper treatment of churns. If churns are carelessly treated there will be deterioration of the wood due to the direct action of microorganisms and to the action of products produced by them, and this deterioration is probably more serious than that resulting from the proper care.

Undoubtedly, it is easier to reduce the organisms in a churn to a comparatively small number if the churn is clean than if it is highly contaminated, and this indicates the importance of properly cleaning the churn regularly. The cleaning must involve the removal of the milk solids as completely as possible because of the tendency of these materials to protect organisms, to favor the growth of organisms and to work into cracks and crevices in the wood from which their removal is practically impossible; the penetration of fat into the crack between two staves of an old churn is shown in fig. 12.

There are various methods of treatment that maintain a satisfactory microbiological condition in churns. The efficiency with which the details of a procedure are carried out is probably of more importance than the general procedure used. There

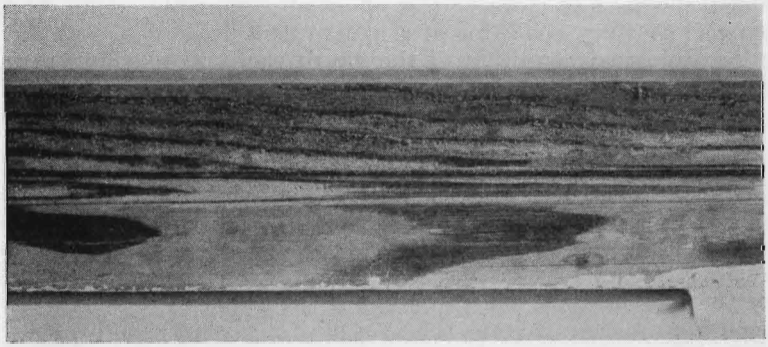

Fig. 12. Part of a stave from an old churn showing the penetration of fat between the staves; the penetration of paint from the outside of the churn is also evident.

are, however, certain principles involved in the care of churns that should be definitely recognized with any method. The churn must be thoroughly washed, some procedure for the destruction of the organisms that are reasonably susceptible to destruction must be followed, and then the growth of the remaining organisms must be controlled and excessive contamination from the air prevented. The prevention of the growth of organisms in the churn is simplified if the churn is so treated that it will dry rapidly. It should be recognized that a churn in good mechanical condition presents less of a problem than one in a poor mechanical condition.

CONCLUSIONS

PART 1

Agar disc counts on 27 churns in commercial use in 24 Iowa butter plants showed that the churns varied widely in their microbiological condition, some of them containing comparatively small numbers of organisms while others contained excessive numbers. Bacteria were regularly much more numerous in the churns than yeasts or molds, and molds were usually somewhat more numerous than yeasts. In general, when the bacterial counts were low, comparatively few species were present and organisms of the genus Bacillus and micrococci predominated while, when the bacterial counts were high, many species were commonly present with yellow micrococci usually predominating.

The washing procedures reported for some of the churns were very evidently inadequate and, moreover, some of the churns were left with considerable moisture in them. In general, the moist churns had more or less of an objectionable odor. The general sanitary condition of a plant was a better index of the microbiological condition of the churn than the washing procedure reported.

PART 2

Agar disc counts over extended periods, on two churns that were regularly given a thorough treatment with hot water after thorough washing showed that, although there was some variation in the numbers of organisms present, the numbers were commonly comparatively small. Bacteria were more numerous in the churns than yeasts or molds, and usually molds were somewhat more numerous than yeasts. In general, the bacteria were of few types and were largely members of the genus Bacillus.

PART 3

The use of a solution of sodium hypochlorite, chlorinated lime or calcium hypochlorite on churns treated with hot water resulted in very definite reductions in the numbers of bacteria present, as measured by agar disc counts; there were also reductions in the numbers of yeasts and molds, but the original counts of these organisms were so low that the changes in counts were not conspicuous.

The use of a cold, saturated sodium chloride solution on a churn treated with hot water did not result in reductions in the numbers of bacteria, yeasts or molds, as determined by agar disc counts.

PART 4

The treatment of highly contaminated churns with hot water effected striking reductions in the numbers of organisms present as determined by either the rinse or agar disc method. The yeasts, molds and other non-resistant types were very largely eliminated, but there were considerable numbers of resistant bacteria, especially Bacillus types, present after treatment. The hot water used for the treatment always contained considerable numbers of bacteria.

The treatment of highly contaminated churns with a solution of either sodium hypochlorite or a chloramine preparation regularly resulted in large reductions in the numbers of organisms present. After exposure to the churns the hypochlorite solution contained comparatively small numbers of organisms while the solution of the chloramine preparation contained considerable numbers.

PART 5

Agar plates exposed near a churn showed that bacteria, yeasts and molds were falling from the air in considerable numbers. Plates exposed in churns with the doors on the sides indicated that organisms were also falling inside the churns, but in smaller numbers than outside. Both outside and inside the churns, bacteria were falling in larger numbers than the yeasts or molds and the molds in larger numbers than the yeasts.

In comparisons, in which one set of plates was exposed in a churn with a muslin door covering while another set was exposed in an unprotected churn, the numbers of organisms falling in the protected churn were less than in the unprotected churn. The decrease was especially striking with the bacteria.

PART 6

General observations at the Iowa Agricultural Experiment Station on the contamination from churns indicate that serious

contamination of butter sometimes occurs from churns that are being treated carefully. In one such instance that was studied in detail, a loose shelf support was apparently responsible for the contamination since material under the support had a very objectionable odor and contained large numbers of bacteria; the replacement of the support with one that could be held firmly in place resulted in a great reduction in the contamination from the churn.

PART 7

Each of 61 pure cultures of bacteria isolated from churns brought about a change in unsalted butter stored at 59° F., although with a small number of them the change was slight. In general, the changes produced by the non-spore forming rods and the micrococci were more rapid and more extensive than those produced by the Bacillus types. Mixed cultures produced marked changes, the deterioration of the butter generally being very rapid.

Plate and microscopic counts on fresh unsalted butter from clean and from highly contaminated churns showed that the contaminated churns commonly contributed considerable numbers of bacteria to the butter. Bacterial development was usually more extensive in the butter from the contaminated churns than in that from the clean churns at 45° F. and also at 70° F.

There were no significant differences in the keeping qualities of salted butter from clean and from contaminated churns at either 32° or 45° F. The unsalted butter from clean churns showed keeping qualities distinctly superior to those of the unsalted butter from contaminated churns, both at 32° F. and at 45° F. The unsalted butter deteriorated much more rapidly than the salted butter, both at 32° and 45° F., and the deterioration was more rapid at 45° F. than at 32° F. Rancidity was the most common defect that developed in the unsalted butter from contaminated churns, and cheesiness was the most common defect appearing in the unsalted butter from the clean churns.

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